Aged Garlic Extract™ Research 2020

Experts from Peer Reviewed Scientific Journals & Meetings

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Aged Garlic Extract™

Research Excerpts from Peer Reviewed Scientific Journals & Scientific Meetings

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AGED GARLIC EXTRACT INTRODUCTION

Kyolic® Aged Garlic Extract™ is the only true odorless garlic supplement in the market. The production of Kyolic Aged Garlic Extract begins with garlic grown on Wakunaga farms under strictly controlled organic conditions without the use of chemical fertilizers, herbicides or pesticides. Wakunaga's garlic crop is cultivated with time-tested procedures to enhance its beneficial constituents. Quality control begins when the harvested raw garlic is tested for conformity to specified quality standards. Only the finest garlic is used for the production of Kyolic. The unique aging process applied in manufacturing Aged Garlic Extract distinguishes Kyolic from other garlic products available on the market. First, the garlic cloves are cleaned and sliced. Then, under carefully controlled conditions, the sliced garlic is stored in an aqueous solution in stainless steel tanks and naturally aged, without heating, for up to 20 months. Through this unique process, the harsh and unstable organosulfur compounds are converted into mild and effective compounds including sulfur-containing amino acids (e.g. *S*-allylcysteine, *S*-allylmercaptocysteine) and Maillard reaction compounds that are responsible for Kyolic's health benefits. Aged Garlic Extract increases in the antioxidant value during the natural aging process. This conversion eliminates odor-causing components, resulting in a truly odorless Kyolic Aged Garlic Extract that contains safe, stable, bioavailable and beneficial compounds. Kyolic Aged Garlic Extract is manufactured under ISO 9001 quality control and Good Manufacturing Practices (GMP) as detailed in The Code of Federal Regulations. Aged Garlic Extract also complies with the specifications established in the US Pharmacopeia/National Formulary (USP/NF) monograph. Aged Garlic Extract, and its various constituents, has been the subject of more than 800 scientific publications around the world including Japan, the United States and Europe since its development in 1955.

The Kyolic Difference

Organic Farming

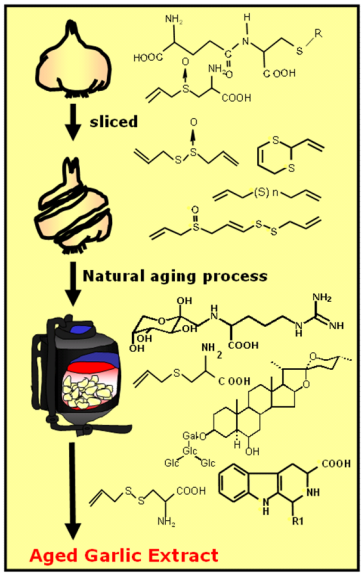
Natural Aging in Stainless Steel Tanks

Natural Aging Process

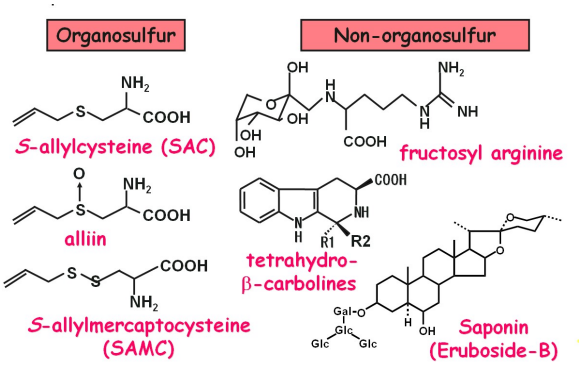
Organically Grown Garlic are sliced and soaked in Stainless Steel Tanks

- Extraction (up to 20 months)
- Bioconversion
  (Natural Enzymatic Reactions)
- Increase in Water-Soluble Compounds
  e.g. *S*-allylcysteine (SAC)
- Harsh and Irritating Compounds
- Converted into Safe and Beneficial Compounds

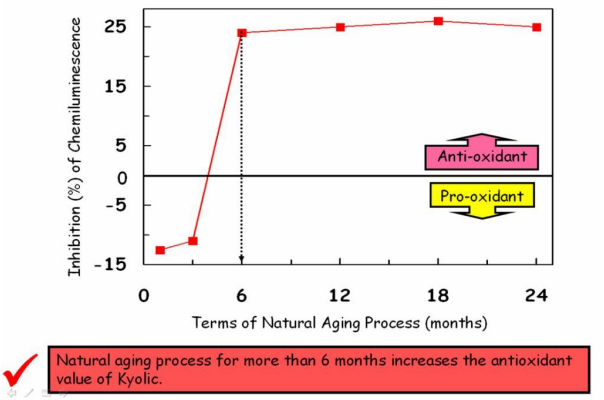
Aged Garlic Extract



Reported Bioactive Compounds in AGE



Aging Process Makes Garlic Extract an Antioxidant



Progress of Published Scientific Research Papers on AGE and its Constituents

2019: More than 850 papers!!

Aged Garlic Extract™

Research Excerpts from Peer Reviewed Scientific Journals & Scientific Meetings

PHARMACOLOGICAL PROPERTIES OF AGED GARLIC EXTRACT

Cardioprotective Effects Seen in Clinical Studies

Inhibits Plaque Formation in the Coronary Artery

Budoff M, Takasu J, et al. 2004. Prev Med. 39(5):985-91.

Budoff M. 2006. J Nutr. 136(3 Suppl):741S-4S.

In a placebo-controlled, double-blind, randomized clinical study, patients with known coronary artery disease who were given 4 ml of Kyolic AGE Liquid a day for one year have shown a significantly lower calcium score (mean change: 7.5 ± 9.4%) than the placebo group (22 ± 18.5%) using electron beam computed tomography (EBCT). Approximately 65% significant inhibition in plaque formation was observed in the Kyolic group compared to placebo.

Ahmadi N, Hajsadeghi F, et al. 2008. Arteriosclerosis, Thombosis and Vascular Biology Annual Conference. Atlanta, GA. Apr 16-18. Abst #P484.

Budoff M, Ahmadi N, et al. 2008. Quality of Care and Outcomes Research in Cardiovascular Disease and Stroke. Baltimore, MD. Apr 30-May 2. Abst #98.

Budoff MJ, Ahmadi N, et al. 2009. Prev Med. 49(2-3):101-7.

Ahmadi V, Tsimikas S, et al. 2010. Am J Cardiol. 105(4):459-66.

Intermediate-risk patients for cardiovascular disease were treated with 4 capsules of Kyolic Formula 108 (AGE+S) (each capsule containing AGE [250 mg], vitamin B_{12} [100 mcg], folic acid [300 mcg], vitamin B_6 [12.5 mg], and L-arginine [100 mg]) daily for 1 year in a placebo-controlled, double-blind, randomized trial. After 1 year, coronary artery calcium (CAC) progression was significantly lower by 78%, Post-cuff Deflation Area Under the Temperature Curve (TMP-AUC) (P=0.02) and temperature rebound (TR) were significantly higher in the AGE+S group compared to placebo. Total cholesterol, low-density lipoprotein cholesterol (LDL-C), homocysteine, immunoglobulin G (IgG), immunoglobulin M (IgM) autoantibodies to malondialdehyde (MDA)-LDL and apolipoprotein B (apoB)-immune complexes were decreased, whereas high-density lipoprotein (HDL), oxidized phospholipids (OxPL) on apoB-100 (OxPL/apoB) and lipoprotein (a) [Lp (a)] were significantly increased in AGE+S to placebo.

Ahmadi N, Zeb I, et al. 2010. American Heart Association Scientific Sessions 2010 Archive. Chicago, IL. Nov 13-17. Abst #20824.

The same study showed a strong correlation between increase in CAC scores and epicardial (EAT) (p=0.0001), peri-aortic (PaAT) (p=0.008) and subcutaneous adipose tissue (SAT) (p=0.01) from baseline to 12 months. At 1 year, CAC progression and increase in EAT, pericardial (PAT), PaAT and SAT were significantly lower in the AGE+S as compared to the placebo group ($p<0.05$). This study documents that AGE+S is associated with reducing the rate of progress in coronary atherosclerosis and adipose tissue.

Ahmadi N, Larijani VN, et al. 2012. American College of Cardiology ACC.12. Chicago, IL. Mar 24-27. Presentation #1112-571.

Metabolically active epicardial adipose tissue (mEAT) reduced in AGE+S as compared to placebo (p=0.003) from baseline to 12 months. Strong correlation between decrease in mEAT and lack of CAC progression (p=0.0001) was noted. The adjusted risk of reduced mEAT was 7.69 (p=0.0001) and the likelihood ratio of combined lack of CAC progression and reduction in mEAT was 12.82 (p=0.0001) folds higher in AGE+S as compared to placebo.

Ahmadi N, Zughaib H, et al. 2011. American Heart Association & American Stroke Association Scientific Sessions. Orlando, FL. Nov 12-16.

A significant correlation was noted between increase in CAC and decreases in bone mineral density (BMD) (p=0.0001). At 1 year, the mean increase in CAC and decrease in BMD was significantly lower in the AGE+S as compared to the placebo group ($p<0.05$). After adjustment of conventional risk factors, the risk of CAC progression and reduced BMD was 65% and 68% less in AGE+S as compared to the placebo cohort ($p<0.05$).

Ahmadi N, Nabavi V, et al. 2013. Int J Cardiol. 168(3):2310-4.

Subjects taking 4 capsules of Kyolic Formula 108 (AGE-S) (each capsule containing AGE 250 mg, vitamin B_{12} 100µg, folic acid 300 µg, vitamin B_6 12.5 mg and L-arginine 100 mg) from baseline to 12 months showed a strong correlation between increase in white epicardial adipose tissue (wEAT) and coronary artery calcium (CAC) ($r^2=0.54$, p=0.0001). At 1 year, the risks of CAC progression and increased wEAT and homocysteine were significantly lowered in AGE-S to placebo ($p<0.05$). Similarly, brown epicardial adipose tissue (bEAT) and temperature-rebound were significantly higher in the AGE-S as compared to placebo ($p<0.05$). A strong association was noted between increase in temperature-rebound and bEAT/wEAT ratio ($r^2=0.08$, p=0.001), which was more robust in AGE-S.

Larijani VN, Zeb I, et al. 2011. American College of Cardiology ACC.11. New Orleans, LA. Apr 2-5.

Zeb I, Ahmadi N, et al. 2011. J Cardiovasc Comput Tomo. SCCT 6th Annual Scientific Meeting. Denver, CO. Jul 14-17. 5:S43.

Zeb I, Ahmadi N, et al. 2012. American College of Cardiology.

In a placebo-controlled, double-blind, randomized trial, 65 intermediate risk firefighters were treated with 4 capsules of Kyolic Formula 110 containing AGE and Coenzyme Q10 (CoQ10) (AGE+CoQ10, 1200 mg and 120 mg, respectively) daily for 1 year. Mean coronary artery calcium (CAC) progression was significantly lower in the AGE+CoQ10 (p=0.01) than placebo. Oxidized phospholipids (OxPL)/apolipoprotein B-100 (apoB) and lipoprotein (a) [Lp (a)] were significantly increased in AGE+CoQ10 compared to placebo ($p<0.05$). There was a significant decrease in auto-antibodies to apoB-immune complexes and malondialdehyde (MDA)-low density lipoproteins (LDL) in auto-antibodies to

ACC.12. Chicago, IL. Mar 24-27. Presentation #1112-575.

Zeb I, Ahmadi N, et al. 2012. J Cardiovasc Dis Res. 3(3):185-90.

Larijani VN, Ahmadi N, et al. 2013. Nutrition. 29(1):71-5.

Larijani VN, Hajsadeghi F, et al. 2012. American Heart Association (AHA) Scientific Sessions. Los Angeles, CA. Nov 3-7.

apoB-immune complexes and MDA-LDL in AGE+CoQ10 group ($p<0.05$). Similarly, the test for lipoprotein-associated phospholipase A_2 (Lp-PLA_2) (PLAC) and C-reactive protein (CRP) was significantly decreased in AGE+CoQ10 compared to placebo ($p<0.05$). After adjustment for age, gender, conventional cardiac risk factors and statin therapy, AGE+CoQ10 was associated with 3.99 fold lack of CAC progression and 7.0 fold decrease in PLAC compared with placebo.

At 1-year follow-up, perceived mental stress score (PMSS) reduction was significantly higher in AGE+CoQ10 compared to placebo (-0.6% vs. 14.7%, p=0.001). After adjustment for risk factors, AGE+CoQ10 was independently associated with significant reduction in PMSS, C-reactive protein (CRP) and coronary artery calcium (CAC) ($p<0.05$). In which, the likelihood of decrease in PMSS was 84% higher with AGE+CoQ10 and decrease in PMSS in response to AGE+CoQ10 was significantly associated with decrease in CRP and CAC ($p<0.05$).

Ahmadi N, Nabavi V, et al. 2015. Int J Cardiovasc Res. 4:3.

Sixty subjects received either 4 capsules of Kyolic Formula 108 (AGE-S) (AGE 1000 mg, Vitamin B12 400µg, folic acid 1200 µg, Vitamin B6 50 mg and L-arginine 400 mg) or placebo daily for 1 year. The mean increase in coronary artery calcium (CAC) and decrease in bone mineral density (BMD) was significantly lower in the AGE-S as compared to the placebo ($P<0.05$) at 1-year. CAC progression and reduced BMD was 65% and 68% less, in AGE-S, respectively, as compared to placebo ($P<0.05$), after adjustment for risk factors. From baseline to 12 months, a significant correlation was noted between increase in CAC and decreases in BMD. Similarly, a significant correlation was noted between increase in postcuff deflation temperature-rebound (TR) and decrease in homocysteine as well as increase in lipoprotein A (Lp-a) with lack of decrease in BMD. The maximum beneficial effect of AGE-S was noted with increase in TR, lack of decrease in BMD and lack of progression of CAC.

Matsumoto S, Nakanishi R, et al. 2016. J Nutr. 146(2):427S-432S.

Fifty-five patients with metabolic syndrome (MetS) consumed 2400 mg of AGE or placebo daily for 52 weeks. Low-attenuation plaque (LAP) was significantly reduced in the AGE group compared with the placebo group (-1.5% ± 2.3% compared with 0.2% ± 2.0%, P= 0.0049).

Zeb I, Ahmadi N, et al. 2018. Coron Artery Dis. 29(4):325-8.

In a randomized, double-blind, placebo-controlled trial, 60 participants with intermediate risk score and coronary artery calcium (CAC) more than 30% received 4 capsules of Kyolic Formula 108 (AGE + S) (AGE 1000 mg, Vitamin B12 400µg, folic acid 1200 µg, Vitamin B6 50 mg and L-arginine 400 mg) or placebo daily for 1 year. The increase in epidcardial adipose tissue (EAT), pericardial adipose tissue (PAT), periaortic adipose tissue (PaAT), and subcutaneous adipose tissue (SAT) was significantly lower in the AGE + S as compared with the placebo group ($P < 0.05$). The odds ratios of increase in EAT, PAT, PaAT, and SAT were 0.63, 0.72, 0.81, and 0.87, respectively, compared with the placebo group, which even remained significant (all $P < 0.05$) after adjustment for cardiovascular risk factors and statin therapy and BMI.

Shaikh K, Kinninger A, et al. 2020. Exp Ther Med. 19(2):1457-61.

In a double-blind, placebo-controlled, randomized trial, patients with diabetes mellitus (DM) were assigned to consume 2400 mg AGE per day or placebo for 1 year. Compared to the placebo group, the AGE group exhibited a statistically significant regression in normalized low-attenuation plaque (LAP) [median and standard deviation (SD) -0.2 (18.8) vs. 2.5 (69.3), P=0.0415].

Hutchins E, Shaikh K, et al. 2020. Exp Ther Med. 19(2):1468-71.

The same study above showed a trend towards a significant reduction in left ventricular myocardial mass (LVM) at follow-up as compared to baseline in the AGE group (119.30±34.77 vs. 121.0±34.70, P=0.059). No change was observed in the LVM in the placebo group at 1-year follow-up as compared to baseline (124.6±37.33 vs. 124.6±35.13, P=0.9). This study indicated that AGE may decrease or stabilize LVM.

Lipid Lowering Effects

Kawashima Y, Ochiai Y, et al. 1985. Shinryou to Shinyaku (Treat New Med). 26(3):377-88.

After taking 4 capsules of Kyoleopin® (KLE), each day for 16 weeks, a 9% reduction in serum cholesterol (234 ± 10 mg/dL → 213 ± 10 mg/dL), a 17% reduction in triglycerides (229 ± 20 mg/dL → 190 ± 16 mg/dL) and a 9% reduction in low-density lipoprotein (LDL) cholesterol (152 ± 10 mg/dL) → 138 ± 10 mg/dL) were seen in 19 patients.

Lau B, Lam F, et al. 1987. Nutr Res. 7:139-49.

Subjects with elevated levels of cholesterol (220-440 mg/dL) were given 4 capsules of Kyolic AGE Liquid daily or placebo for 6 months. Serum cholesterol and triglycerides levels in the Kyolic group significantly dropped while the placebo group showed no change. After 6 months, 11 of 15 subjects (73%) experienced a significant reduction in serum cholesterol compared to baseline (↓12-31%), while 10 of 15 subjects experienced decreases greater than 10% in triglycerides. Subjects taking Kyolic also showed a decrease in serum levels of low-density lipoprotein (LDL) cholesterol and an increase in high-density

lipoprotein (HDL) cholesterol.

Warshafsky S, Kamer R, et al. 1993. Ann Intern Med. 119(7 Pt 1):599-605.

A meta-analysis conducted by Warshafsky et al. (1993) included Lau's paper (1987) in the pooled data and found a 9% reduction in cholesterol from garlic intake.

Silagy C, Neil A. 1994. J Royal Coll Physic Lond. 28(1):39-45.

Lau's paper (1987) met the stringent criteria established for the meta-analysis of various clinical studies conducted by Silagy et al. (1994) to determine the effects of garlic on cholesterol. Pooling Lau's data in with a total of 16 trials including more than 900 subjects confirmed that garlic-treated subjects experienced a 12% reduction in cholesterol beyond of the effect of placebo alone.

Steiner M, Lin RI. 1994. J Am Coll Nutr. 13(5):524.

Steiner M, Khan AH, et al. 1996. Shinyaku to Rinsho (Jpn J New Remedies Clin). 45(3):456-66.

In a 6-month, double-blind crossover study, AGE supplementation led to a modest reduction in total cholesterol of 8% in 2/3 of men with high cholesterol levels (230-290 mg/dL) on a low-fat/low cholesterol diet (NCEP Step I diet). In participants who had good compliance in their regimes, triglycerides were significantly reduced by 18.9% compared to baseline and 6.7% compared to placebo.

Steiner M, Khan AH, et al. 1996. Am J Clin Nutr. 64(6):866-70.

In this double-blind, crossover study of hyperlipidemic patients (cholesterol 220-290 mg/dl) on the National Cholesterol Education Program (NCEP) Step I diet, a 6.1% reduction in total serum cholesterol and 4.6% reduction in low-density lipoprotein (LDL) cholesterol was found following 6-month oral intake of AGE compared to placebo.

Yeh YY, Lin R, et al. 1995. Am J Coll Nutr. 13(5):545. Abst #83.

Yeh YY, Lin R, et al. 1997. In: Food Factors for Cancer Prevention. Springer-Verlag, pp. 226-30.

In a 5-month double-blind, randomized, placebo-controlled intervention study in men with high cholesterol (241 ± 21 mg/dL) maintaining their normal diets, Kyolic AGE was found to reduce total plasma and low-density lipoprotein (LDL) cholesterol by 7% and 10%, respectively.

Circulation-Enhancing/Blood-Thinning Clinical Effects

Kikuchi N, Nishimura Y, et al. 1994. Shinyaku to Rinsho (Jpn J New Remedies Clin). 43(1):146-58.

Via skin temperature thermography, Leopin-5® (LE-5) showed a greater enhancement in microcirculation than cooked garlic juice in patients who demonstrated symptoms of deficient microcirculation and various symptoms associated with insufficient microcirculation such as headaches, dizziness, vertigo, weariness, lumbago, etc., were also improved.

Okuhara T. 1994. Jpn Pharmacol Therapeut. 22(8):3695-701.

Also using skin temperature thermography, following both single and continuous administration of 2 capsules of Kyolic AGE Liquid (0.8 ml per capsule) for 14 days showed a greater degree of improvement in blood flow in subjects.

Steiner M, Lin RI. 1994. J Am Coll Nutr. 13(5):524.

Steiner M, Khan AH, et al. 1996. Shinyaku to Rinsho (Jpn J New Remedies Clin). 45(3):146-58.

AGE supplementation led to a significant reduction in platelet adhesion to fibrinogen by 34-58% and a 10-25% reduction in platelet aggregation in men with high cholesterol levels (230-290 mg/dL) on a low-fat/low cholesterol diet (National Cholesterol Education Program or NCEP).

Okuhira M, Holbert H, et al. 2000. 3rd International Congress on Phytomedicine. Munich, Germany. Oct 11-13. Phytomedicine. 7(2):49. Abst #SL-101.

A double-blind, randomized intervention study was conducted using 4 ml of AGE daily in healthy subjects for 36 weeks. Significant differences were noted in the level of high-density lipoprotein (HDL) cholesterol which was increased in response to AGE, and in platelet aggregation induced by epinephrine and platelet adhesion to fibrinogen both were inhibited by AGE.

Steiner M, Li W. 2001. J Nutr. 131(3 Suppl):980S-4S.

AGE administration of 2.4 and 4.8 g/day given to moderately hypercholesterolemic men in a randomized, double-blind clinical trial. AGE reduced platelet aggregation induced by collagen and epinephrine, and inhibited its adhesion to fibrinogen and collagen. In addition, blood pressure was reduced by AGE.

Steiner M, Lin RIS. 1998. J Cardiovasc Pharmacol. 31(6):904-8.

In a 10-month placebo-controlled study on men with high cholesterol were supplemented with 7.2 g of AGE or placebo. Blood samples were drawn and epinephrine and collagen were added to induce aggregation or clumping. Blood samples from the AGE group showed 33% less platelet aggregation after 3 months and 43% less aggregation after 6 months when compared to the placebo group. The ability of platelets adhering to fibrinogen, a clotting agent in blood, was reduced by approximately 30% in subjects taking AGE compared to placebo.

Rahman K, Billington D. 2000. J Nutr. 130(11):2662-5.

Dillon S, Lowe G, et al. 2000. 3rd International Congress on

Kyolic AGE (5 ml) given daily to subjects with normal cholesterol for 13 weeks in a randomized, double-blind study reduced clotting as seen by a significant inhibition of the ability of adenosine diphosphate (ADP), a clotting agent, to cause platelet aggregation. Both the total percentage and initial rate of clotting

Phytomedicine. Munich, Germany. Oct 11-13. Phytomedicine. 7(2):49. Abst #SL-99.

were significantly inhibited.

Mizuno I, Sumioka I, et al. 2004. Oyo Yakuri (Pharmacometrics). 67(3/4):371-8.

Leopin Royal® (LER) improved the condition of peripheral blood circulation by a 4-week human trial, where 1 ml of LER was taken twice a day after meals in the morning and evening. The condition of the peripheral blood circulation was estimated 0, 2, and 4 weeks after taking LER. LER was found to improve the peripheral blood circulation index and the width of small veins.

Williams MJA, Sutherland WHF, et al. 2005. Phytother Res. 19(4):314-9.

A randomized, placebo-controlled, crossover design study supplemented 15 men with AGE. All men had angiographically proven coronary artery disease (CAD) treated with aspirin and statin. When supplemented with AGE, brachial artery flow-mediated endothelium-dependent dilation (FMD) significantly increased (44%, P=0.04). At the end of AGE treatment, FMD levels were significantly higher (P=0.03) when compared with the placebo treatment.

Abahji T, Ide N, et al. 2005. 5th International Conference on Homocysteine Metabolism. Mano, Italy. Jun 26-30. Haematologica Reports. 1(3):39. Abst # P036.

Weiss N, Ide N, et al. 2006. J Nutr. 136(3 Suppl):750S-4S.

In a placebo-controlled, blinded, crossover pilot study, pretreatment with AGE for 6 weeks significantly reduced the adverse effects associated with acute hyperhomocysteinemia in macro- and microvascular endothelial dysfunction. AGE may be partly responsible in preventing a decrease in bioavailable nitric oxide (NO) and endothelium-derived hyperpolarizing factor during acute hyperhomocysteinemia.

Abahji T, Weiss N. 2005. Med Report. 31:11.

Weiss N, Abahji T, et al. 2006. XIV International Symposium on Atherosclerosis. Rome, Italy. Jun 18-22. Abst # Tu-P7:143.

Hyperhomocysteinemia leads to endothelial dysfunction and decreased bioavailable nitric oxide (NO) due to increased vascular oxidant stress. Subjects were involved in a placebo-controlled, double-blind crossover study where their brachial artery was studied by ultrasound and laserdoppler fluxmetry (acetylcholine-induced skin profusion) with treatment of AGE before and after 6 weeks of treatment. It was found that AGE improves endothelium dysfunction with the increase in intracellular thiol antioxidants.

Durak I, Atmaca Y, et al. 2007. Vasc Dis Prev. 4(4):478-80.

AGE was given to patients with severe atherosclerosis (1 ml/kg body weight; approximately 0.2 g dry garlic/kg body weight) for 6 months. AGE consumption resulted in significant increases in plasma nitric oxide synthase (NOS) activity and nitric oxide (NO) levels of atherosclerotic patients.

Smith S, Rahman K, et al. 2010. International Conference on Hypertension, Lipids, Diabetes and Stroke Prevention. Berlin, Germany. Mar 4-6, p. 231.

Volunteers were asked to ingest 5 ml of AGE per day for 14 days. Blood samples were taken at the beginning of the trial, after 14 days of AGE ingestion and again a further 2 weeks of a wash out period. AGE was found to inhibit platelet aggregation and intraplatelet cyclic adenosine monophosphate (cAMP) was increased after 2 weeks of AGE ingestion.

Wlosinska M, Nilsson AC, et al. 2019. Int Wound J. 16(6)::1487-93.

A total of 122 patients with Framingham Risk Score ≥ 10 were randomized in a double-blinded manner to placebo or 2400 mg AGE daily for 1 year. Cutaneous microcirculation was measured at 0 and 12 months using laser Doppleri velocimetry. The mean percent change between the two time points 0 and 12 months was 102, 64 (174, 15)% change for AGE and 78, 62 (107, 92)% change for the placebo group ($F[1, 120] = 5.95$, $P < 0.016$), 12 months of AGE increases the microcirculation in patients with an increased risk for cardiovascular events estimated using the Framingham risk score. Increased microcirculation could hypothetically facilitate wound healing.

Hamal S, Cherukuri L, et al. 2020. Exp Ther Med. 19(2):1485-9.

Sixty-five subjects with type 2 diabetes mellitus (T2DM) were enrolled and randomized to AGE or placebo group over a 3-month period in a double-blind placebo controlled trial. In the AGE group, cardio-ankle vascular index (CAVI) was reduced on average by 0.71±1.27 vs. a mean reduction of 0.13±0.94 in the placebo group (P=0.04). This study demonstrates that AGE has a positive impact on endothelial function in patients with T2DM and may play a role in the primary prevention of cardiovascular disease.

Hypotensive/Blood Pressure Lowering Effects

Steiner M, Khan AH, et al. 1996. Shinyaku to Rinsho (Jpn J New Remedies Clin). 45(3):456-66.

Compared to baseline values, significant reductions in both systolic and diastolic blood pressure (9% decreases for both) were noted in this double-blind, crossover study. Hyperlipidemic patients were consuming the National Cholesterol Education Program (NCEP) Step I diet given placebo for 6 months and followed by AGE for 4 months showed the most significant results.

Steiner M, Khan AH, et al. 1996. Am J Clin Nutr. 64(6):866-70.

Another double-blind, placebo-controlled crossover study of hyperlipidemic patients (with cholesterol levels of 220-290 mg/dL) on the National Cholesterol Education Program (NCEP) Step I diet showed a 5.5% decrease in systolic blood pressure following a 6-month oral intake of AGE.

Tokunaga A, Hayashi T. 1996. Shinyaku to Rinsho (Jpn J New Remedies Clin). 45(10):2015-20.

Patients with high and normal blood pressure were given 6 ml of Leopin-5® (LE-5) for 8 days. LE-5 was effective in lowering blood pressure in those with high blood pressure, but was not effective in those with normal blood pressure.

Ried K, Frank OR, et al. 2010. Maturitas. 67(2):144-50.

In a parallel, randomized, double-blind, placebo-controlled study, patients with treated but uncontrolled hypertension (systolic blood pressure [SBP] ≥ 140 mm Hg or diastolic blood pressure [DBP] ≥ 90 mm Hg) were given 4 capsules of Kyolic AGE (960 mg containing 2.4 mg of *S*-allylcysteine [SAC]) or placebo daily for 12 weeks. Patients taking AGE for 12 weeks were shown to have lower systolic blood pressure, on average 10.2 ± 4.3 mm Hg (p=0.03), than the control group.

Ried K, Frank OR, et al. 2013. Eur J Clin Nutr. 67(1):64-70.

Patients with uncontrolled systolic hypertension (systolic blood pressure [SBP] ≥ 140 mm Hg) participated in a double-blind, randomized, placebo-controlled dose-response trial of 12 weeks. Participants were allocated to one, two or four capsules daily of Kyolic AGE (240/480/960 mg containing 0.6/1.2/2.4 mg of *S*-allylcysteine [SAC]) or placebo. Mean SBP was significantly reduced by 11.8 ± 5.4 mm Hg in the garlic-2-capsule-group over 12 weeks compared with placebo (P=0.006), and reached borderline significant reduction in the garlic-4-capsule-group at 8 weeks (-7.4 ± 4.1 mm Hg, P=0.07).

Ried K, Travica N. et al. 2015. The 5th Science of Nutrition in Medicine and Healthcare Conference. Melbourne, Australia. May 2-3.

Ried K, Travica N, et al. 2016. Integr Blood Press Control. 9:9-21.

Patients with uncontrolled hypertension were given 1.2 g of AGE (containing 1.8 mg *S*-allylcysteine) or placebo daily in a double-blind, randomized, placebo-controlled trial of 12 weeks. Mean blood pressure was significantly reduced by 5.0 ± 2.1 mmHg (p=0.016) systolic, and in responders by 11.5 ± 1.9 mmHg systolic and 6.3 ± 1.1 mmHg diastolic compared to placebo (p<0.001). Central blood pressure, central pulse pressure, mean arterial pressure, augmentation pressure, pulse wave velocity and arterial stiffness tended to improve in the garlic-group more than in the placebo group. Trends in beneficial effects of garlic on tumor necrosis factor-α (TNF-α), total cholesterol, low-density lipoprotein (LDL) cholesterol and apolipoproteins were also observed.

Ried K, Travica N, et al. 2018. Front Nutr. 5:122.

Participants with uncontrolled hypertension completed a double-blind randomized placebo-controlled trial of 12 weeks. The group who consumed 1.2 g of Kyolic AGE daily shown a significant reduction in mean blood pressure by 10 ± 3.6 mmHg systolic and 5.4 ± 2.3 mmHg diastolic compared to placebo. AGE also significantly lowered central blood pressure, pulse pressure and arterial stiffness ($p < 0.05$).

Gruenwald J, Bongartzz U, et al. 2020. Exp Ther Med. 19(2):1490-9.

This randomized double-blind, placebo-controlled clinical trial examined 57 subjects with slightly elevated blood pressure over a period of 12 weeks. A significant decrease in blood pressure in the AGE group, and in particular diastolic blood pressure. Using EndoPAT™ technology, the augmentation index (AI) was analyzed, which measures arterial stiffness (lower AI values reflect better arterial elasticity). The AGE group exhibited a significant improvement in arterial elasticity, measured as AI75, by 21.6%.

Sickle Cell Anemia

Takasu J, Uykimpang R, et al. 2002. BMC Blood Disorders. 2(1):3.

Unanimously, the patient's count of Heinz bodies decreased from 58.9% to 29.8% during the 4 weeks of the study.

Ohnishi ST. 2001. Therapeutic Use of Specially Processed Garlic for Sickle Cell Disease. US Patent #6,254,871 B1.

Kyolic AGE is patented by the U.S. government for its ability to reduce painful crisis of sickle cell anemia.

Metabolic Syndrome

Gomez-Arbelaez D, Lahera V, et al. 2013. Mediators Inflamm. 285795.

In a double-blind, crossover, randomized, placebo-controlled clinical trial, subjects with metabolic syndrome (MS) were given 1.2g/day of Kyolic AGE were given for 24 weeks (12 weeks of AGE and 12 weeks of placebo) increased plasma levels of adiponectin (P=0.027) with no serious side effects.

Decreased Levels of Serum Prostaglandins PGE_2 and $PGF_{2\alpha}$

Dmitrov NV, Bennink MR. 1997. Ch. 21. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 199-202.

In this pilot study, Dimitrov et al. (1997) found that 10 ml of AGE liquid taken daily for three months decreased the levels of the serum prostaglandins PGE_2 and $PGF_{2\alpha}$ in most healthy women.

Cardiovascular Review

Loy MH, Rivlin RS. 2000. Nutr Clin Care. 3(3):145-52.

Most studies on garlic and its effect on cardiovascular health produce positive results (e.g., reduced serum cholesterol, decreased high blood pressure and decreased low-density lipoprotein [LDL] cholesterol). Studies have indicated that fresh/raw garlic is necessary in high doses (5-28 cloves/day) to view health benefits, whereas supplement dosage is much lower.

Rahman K. 2001. J Nutr. 131(3 Suppl):977S-9S.

AGE may play an important role in the prevention of cardiovascular disease as demonstrated by many studies (lower serum cholesterol, triglyceride, low-density lipoprotein [LDL] cholesterol and blood pressure levels, and inhibit platelet aggregation).

Banerjee SK, Maulik SK. 2002. Nutrition J. 1:1-14.

Many garlic articles, including scientific papers using AGE, have shown benefits in cardiovascular health, but depending upon the preparation.

Rahman K, Lowe GM. 2006. J Nutr. 130(3 Suppl):736S-40S.

Garlic has multiple effects on parameters involved in cardiovascular disease, as confirmed by numerous studies. AGE has shown to have cardioprotective effects in a number of trials. Dietary supplementation with AGE decreased plasma and urinary oxidative stress marker levels in smokers and non-smokers and increased the antioxidant status of smokers. Thus, AGE may prevent or delay free radical mediated diseases such as atherosclerosis.

Liu CT, Sheen LY, et al. 2007. Mol Nutr Food Res. 51(11):1353-64.

Diabetes affects a large segment of the population and the prevalence of this disease is rapidly increasing. The validity of data from previous studies of the hypoglycemic effect of garlic including AGE, in diabetic models and the preventive effects on diabetes complications are discussed in this review. The role of garlic as both an insulin secretagogue and as an insulin sensitizer is reviewed. Evidence suggests that garlic's antioxidative, anti-inflammatory and antiglycative properties are responsible for garlic's role in preventing diabetes progression and the development of diabetes-related complications. Large-scale clinical studies with diabetic patients are warranted to confirm the usefulness of garlic in the treatment and prevention of diabetes.

Zeng T, Guo FF, et al. 2012. J Sci Food Agric. 92(9):1892-902.

A total of 26 studies were included into this meta-analysis. Overall, garlic, including AGE, was superior to placebo in reducing serum total cholesterol (TC) (0.28mmol/L, P=0.001) and triglyceride (TG) (0.13 mmol/L, P<0.001) levels. The effects of garlic were more striking in subjects with long-term intervention and higher baseline TC levels and AGE, specifically, were more effective in reducing serum TC levels.

Cicero AF, Borghi C. 2013. Curr Hypertens Rep. 15(3):260-7.

A relatively large body of evidence supports the use of AGE and other ingredients (potassium, L-arginine, vitamin C, cocoa flavonoids, coenzyme Q10 and controlled-release melatonin) to have possible blood-pressure lowering effect, which are also antioxidant agents with a high tolerability and safety profile.

Ried K, Fakler P. 2014. Integr Blood Press Control. 7:71-82.

Garlic-derived polysulfides, found in AGE, stimulate the production of the vascular gasotransmitter hydrogen sulfide (H_2S) and enhance the regulation of endothelial nitric oxide (NO), which induces smooth muscle cells relaxation, vasodilation, and blood pressure (BP) reduction. There are several dietary and genetic factors that influence the efficiency of the H_2S and NO signaling pathways and may contribute to the development of hypertension. Sulfur deficiency may play a part in the development of hypertension, and could be alleviated with the supplementation of organosulfur compounds derived from garlic.

Shouk R, Abdou A, et al. 2014. Nutr Res. 34(2):106-15.

This review discusses the molecular, biochemical and cellular rationale underlying the antihypertensive properties of garlic and its bioactive constituents, including *S*-allylcysteine (SAC). SAC has been shown in studies to modulate various parameters implicated in the pathogenesis of hypertension, which include oxidative stress, nitric oxide bioavailability, hydrogen sulfide production, angiotensin converting enzyme activity, expression of nuclear factor κB and the proliferation of vascular smooth muscle cells. This suggests that garlic and garlic derived bioactives have significant medicinal properties with the potential for ameliorating hypertension and associated morbidity.

Hom C, Luo Y, Budoff M. ACC.15. American College of Cardiology. 6th Annual Scientific Session & Expo. J Cardiovasc Comput Tomo. March 14-16, 2015. San Diego, CA.

Hom C, Luo Y, Budoff MJ. 2015. J Nutr Food Sci. 55:005. Doi: 10.4172/2155-9600.S5-005.

Four placebo-controlled, double-blind, randomized studies were reviewed on the efficacy of AGE on coronary artery calcium (CAC) progression and blood pressure as compared to placebo. AGE at 1000 mg or the equivalent amount of placebo was given to subjects for 1 year. At 1 year, median CAC progression was significantly lower in the AGE group (10.8, 95% 0.0-30.7, n=106) than the placebo group (18.3, 95% 3.1-34.0, n=103; P=0.0385). AGE was associated with a 1.78 fold (95% 0.320-0.990, P=0.046) lack of CAC progression compared with placebo. The mean change in diastolic blood pressure (DBP) for the AGE group (n=65) was -6.7 (-15.4-2) over 1 year. The placebo group (n=103) demonstrated an average

decrease in DBP of -3.0 (-11.7-5.7), significantly less than the treated cohort (P=0.038).

Cicero AF, Colletti A. 2015. High Blood Press Cardiovasc Prev. 22(3):203-13.

In particular relatively large body of evidence supports the use of nutraceuticals, including AGE, with possible blood pressure lowering effect.

Varshney R, Budoff MJ. 2016. J Nutr. 146(2):416S-421S.

A review of double-blind, randomized, controlled trials and meta-analyses on the effects of garlic supplements on hypertension, hypercholesterolemia, C-reactive protein (CRP), pulse wave velocity (PWV), and coronary artery calcium (CAC) and their side effects were reviewed. Four meta-analyses and 2 original studies showed garlic supplementation reduced blood pressure by 7-16 mm Hg (systolic) and 5-9 mm Hg (diastolic). Total cholesterol was reduced by 7.4-29.8 mg/dL from 8 meta-analyses. AGE showed the most consistent benefits in studies and a few small studies using AGE also showed favorable effects on CAC, CRP, and PWV. Rare adverse reactions with garlic have been documented.

Ried K. 2016. J Nutr. 146(2):389S-396S.

Randomized controlled trials (RCTs) published between 1955 and 2015 on the effects of garlic preparations on blood pressure, cholesterol and immunity were reviewed. A meta-analysis on the effect of garlic on blood pressure included 20 trials with 970 participants, showed a mean ± SE decrease in systolic blood pressure (SBP) of 5.1 ± 2.2 mg Hg ($P < 0.001$) and a mean ± SE decrease in diastolic blood pressure (DBP) of 2.5 ± 1.6 mm Hg ($P < 0.002$) compared with placebo. For hypertensive subjects (SBP/DBP ≥ 140/90 mm Hg) at baseline revealed a larger significant reduction in SBP of 8.7 ± 2.2 mm Hg ($P < 0.001$; $n = 10$) and in DBP of 6.1 ± 1.3 mm Hg ($P < 0.001$; $n = 6$). In regards to garlic and its effect on blood lipids, a meta-analysis included 39 primary RCTs and 2300 adults treated for a minimum of 2 weeks, shown that garlic was effective in reducing total and low-density lipoprotein (LDL) cholesterol by 10% if taken for > 2 months in individuals with slightly elevated cholesterol concentrations [total cholesterol >200 mg/dL (>5.5 mmol/L)]. Garlic has immunomodulating effects by increasing macrophage activity, natural killer cells, and the production of T and B cells. Garlic also has shown to significantly reduce the number, duration, and severity of upper respiratory infections in clinical trials.

Lopez-Jaramillo P. 2016. J Nutr. 146(2):422S-426S.

Adiponectin is an adipocyte-derived hormone that is abundantly present in plasma. Its concentrations are negatively regulated by the accumulation of visceral fat, and clinical studies implicate that hypoadiponectinemia in the pathogenesis of diabetes mellitus type 2, coronary artery disease, hypertension, and left ventricular hypertrophy. In contrast, high concentrations of adiponectin are associated with a decreased risk of coronary artery disease, with an improvement in the differentiation of preadipocytes into adipocytes, and with increased endothelial nitric oxide production. Therefore, adiponectin appears to be an important molecule involved in limiting the pathogenesis of obesity-linked disorders, and may have potential benefits in the treatment and prevention of cardiovascular disease. Caloric restriction, moderate alcohol consumption, consuming a Mediterranean diet can increase adiponectin concentrations. It was also recently reported that AGE and a single food intervention with pistachios can increase adiponectin concentrations in individuals with metabolic syndrome. However, additional studies are needed to evaluate the potential benefits of increasing adiponectin by nutritional interventions in the treatment and prevention of cardiometabolic diseases.

Borghi C, Cicero AF. 2017. Br J Clin Pharmacol. 83(1):163-71.

Studies using dietary supplements or nutraceuticals claiming to show an effect on blood pressure (BP) and published in English from 1990 to 2015 were reviewed. A relatively large body of evidence supports the use of potassium, magnesium, L-arginine, vitamin C, cocoa flavonoids, beetroot juice, coenzyme Q10, controlled-release melatonin and AGE. The antihypertensive effect of all of these nutraceuticals seems to be dose related and the overall tolerability is good.

Ried K. 2020. Exp Ther Med. 19(2):1472-8.

The findings of recent clinical trials investigating the effects of Kyolic AGE on arterial stiffness, and gut microbiota in hypertensive subjects were summarized in this review. The meta-analysis of 12 trials and 553 hypertensive participants (randomized controlled trials published between 155 and December 2018) confirmed that garlic supplements lower systolic blood pressure (SBP) by an average of 8.3±1.9 mmHg and diastolic blood pressure (DBP) by 5.5±1.9 mmHg, similarly to standard anti-hypertensive medications. This reduction in blood pressure was associated with a 16-40% reduction in the risk of suffering from cardiovascular events. Kyolic AGE significantly lowered central blood pressure, pulse pressure, pulse wave velocity and arterial stiffness, and improved gut microbiota. Thus, Kyolic AGE is considered to be a highly tolerable with a high safety profile either as a stand-alone or adjunctive anti-hypertensive treatment, with multiple benefits for cardiovascular health.

We focus on garlic in this review and several other dietary supplements, such as coenzyme Q10, fish oil and probiotics that have exhibited significant beneficial effects on blood pressure in clinical trials. In addition, we discuss the possible mechanisms of action responsible for their anti-hypertensive effects, as well as the safety, active ingredients and their potential use as adjunct therapies for uncontrolled hypertension.

Overview of Cardioprotective Effects of AGE in Clinical Studies

Adiponectin[32]	↑ 10%
Adipose Tissue (AT) around the Heart[33,35]	↓ 48% in total epicardial AT (EAT)
	↑ 23% in brown epicardial AT (bEAT)/white epicardial AT (wEAT) ratio
	↓ 53% in pericardial AT (PAT)
	↓ 71% periaortic AT (PaAT)
	↓ 40% subcutaneous AT (SAT)
Anemia[1]	↓ in 61% of patients
Blood Pressure (Systolic)[2,3,28,31]	↓ 6-10%
Bone Mineral Density (BMD) loss[34]	↓ 84%
Chest Pain[4]	↓ in 90% of patients
Chills (limbs)[4-8]	↓ in 40-100% of patients
Circulation (hands/feet)[8,10]	↑ in 67% of patients
Dizziness[4,5,7,8]	↓ in 50-85% of patients
HDL (good) Cholesterol[26,27]	↑ 7-25%
Headaches[4,5,7,8]	↓ in 50-80% of patients
Homocysteine[23,27]	↓ 18-27%
Heart Palpitations[1,4,5,7,8]	↓ in 45-75% of patients
LDL (bad) Cholesterol[2,11-13,27]	↓ 5-26%
Numbness (limbs)[5,7]	↓ in 40-80%
Oxidized LDL[27]	↓ 35-69%
Oxidative Stress[25]	↓ 29-48%
Plaque Formation in Arteries[22,27,29]	↓ 45-78%
Platelet Adhesion[9,12]	↓ 30-58%
Platelet Aggregation[9,12-21]	↓ 10-25%
Resistance against LDL Oxidation[20]	↑ 100%
Safety with Aspirin and Statins[22,27]	Confirmed
Safety with Warfarin[14,26]	Confirmed
Shortness of Breath[5,7]	↓ in 45-80% of patients
Sickle Cell Anemia[15,19,21]	↓ dense RBCs by 30%
	↓ Heinz bodies by 30%
Susceptibility to LDL Oxidation[9]	↓ 27%
Total Cholesterol[2,3,11-13,17,27]	↓ 6-18%
Triglycerides[3,4,11]	↓ 10-23%
Vascular Function (elasticity)[23,24,27,30]	↑ 15-111%

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Cardioprotective Effects Seen in Pre-Clinical Studies

Lipid Lowering Effects

Qureshi AA, Lin RIS, et al. 1990. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. Aug 28-30, p. 16.

Abuirmeileh N, Yu SG, et al. 1991. FASEB J. 5(6):A1756. Abst #8048.

Yu SG, Qureshi N, et al. 1991. National Conference on Cholesterol and High Blood Pressure. Washington, D.C. Apr 8-10.

AGE and *S*-allylcysteine (SAC) was found to lower total serum cholesterol and low-density lipoprotein (LDL) cholesterol in hypercholesterolemic models. Cholesterol reduction was achieved by inhibition of the activity of key enzymes involved in cholesterol synthesis (β-hydroxy-β-methylglutaryl CoA synthetase and reductase) in the liver. According to Qureshi and Yu et al. (1991), maximum inhibition of cholesterol-producing enzymes activities was observed among the garlic preparations tested in this order:

Kyolic® > SAC > commercial garlic oil > garlic powder

Matsuura H, Slowing K, et al. 2000. Phytomedicine. 7(2):48. Abst #SL-98.

Matsuura H. 2001. J Nutr. 131(3 Suppl):1000S-5S.

The steroid saponins in garlic were isolated and the structures were determined, which exhibit cholesterol-lowering effects. Elevated cholesterol levels were produced in models by feeding them a cholesterol-enriched diet for 16 weeks. A significant reduction in total plasma cholesterol was found in models ingesting the crude glycoside fraction. It was suggested that steroid saponins should be considered active compounds responsible for the cholesterol-lowering effects of garlic and its preparations.

Slowing K, Ganado P, et al. 2001. J Nutr. 131(3 Suppl):994S-9S.

Slowing et al. showed research that indicated that the saponin fraction of garlic reduced serum cholesterol levels and prevented loss of vascular reactivity in models fed with a cholesterol-enriched diet.

Slowing K, Ganado P, et al. 2001. J Nutr. 131(3 Suppl):994S-9S.

Intake of garlic can prevent diet-induced hypercholesterolemia and vascular alterations in the endothelium-dependent relaxation associated with atherosclerosis using a model study system. Models were fed a cholesterol-enriched diet for 16 weeks and were divided into 10 groups. Plasma total cholesterol decreased in all groups treated with garlic and its fractions. Low-density lipoprotein (LDL) cholesterol decreased significantly in the hypercholesterolemic group.

Singh DK, Porter TD. 2006. J Nutr. 136(3 Suppl):759S-64S.

Using chromatography-mass spectrometry, garlic-derived compounds containing an allyl-disulfide or allyl-sulfhydryl group are most likely responsible for decreasing cholesterol synthesis by inhibiting the sterol 4α-methyl oxidase.

Allison GL, Lowe GM, et al. 2006. J Nutr. 136(3 Suppl):782S-8S.

AGE and its constituents inhibit platelet aggregation in a concentration-dependent manner by working synergistically and exerting multiple effects on biochemical pathways. Calcium movement from/into cells will be the key mechanism.

Thomson M, Al-Qattan KK, et al. 2006. J Nutr. 136(3 Suppl):800S-2S.

Raw and boiled aqueous extracts of garlic were administered to models orally and intraperitoneally for 4 weeks. A significant reduction of 11-14% in the cholesterol level of the models was observed in the group that received a low dose of raw aqueous extract of garlic. A significant decrease of 38% in triglyceride levels was also noted in models who received garlic orally and intraperitoneally. Glucose, cholesterol and triglyceride levels were also significantly reduced in models treated with a high dose of raw garlic.

Water-Soluble Sulfur Compounds Best Candidates for Lipid Reduction

Qureshi N, Lin RIS, et al. 1990. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. Aug 28-30, p. 17.

Reductions in both serum and low-density lipoprotein (LDL) cholesterol in both normolipidemic and hypercholesterolemic models were noted. Results were more pronounced for AGE (14-32%) than for commercial garlic powder of garlic oil (11-16%). Significant reductions in triglycerides were also observed following AGE intake and the key enzymes of lipogenesis (acetyl CoA carboxylase and fatty acid synthetase) were significantly inhibited.

Yeh YY, Yeh SM. 1990. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. Aug 28-30, p. 37.

AGE lowered the plasma levels of cholesterol and triglycerides. In models who were fed a diet supplemented with 2% AGE, the plasma triglyceride and cholesterol levels were 30% and 15% lower, respectively, than the control models.

Yeh YY, Yeh SM. 1994. Lipids. 29(3):189-93.

It was found that AGE and one of its key constituents, *S*-allylcysteine (SAC), inhibited the synthesis of cholesterol and fatty acids in cultured liver cells.

Efendy JL, Simmons DL, et al. 1996. J Vasc Res. 33(S1):23. Abst #090.	Eight weeks of Kyolic AGE Liquid significantly reduced elevated levels of β very low-density lipoproteins (βVLDL) induced by cholesterol feeding in models. Elevated βVLDL is a greater risk factor for atherosclerosis than high-density lipoproteins (HDL) or low-density lipoproteins (LDL) in models.
Liu L, Yeh YY. 2000. Lipids. 35(2):197-203. *Yeh YY, Liu L. 2001. J Nutr. 131(3 Suppl):989S-93S.*	The water-soluble organosulfur compounds of garlic: *S*-allylcysteine (SAC), *S*-ethyl cysteine (SEC), *S*-propyl cysteine (SPC), γ-glutamyl-*S*-allyl cysteine, γ-glutamyl-*S*-methyl cysteine, γ-glutamyl-*S*-propyl cysteine and *S*-allylmercaptocysteine (SAMC) inhibited fatty acid synthesis in cultured hepatocytes by 20% to 99% with half maximal inhibitory concentration (IC_{50}) of 0.27 – 1.72 mmol/L. Alliin, *S*-allyl-*N*-acetyl cysteine, *S*-allylsulfonyl alanine and *S*-methylcysteine did not inhibit fatty acid synthesis. All tested water-soluble compounds except SAMC did not alter cellular release of lactate dehydrogenase (LDH) into medium. Lipid-soluble compounds such as diallyl sulfide, diallyl disulfide, diallyl trisulfide, dipropyl sulfide and dipropyl disulfide reduced fatty acid synthesis by 10-98%, which was accompanied by markedly increased LDH release, an indicator of cell toxicity. In addition, SAC and SPC depressed triacylglycerol (TG) and phospholipid (PL) synthesis. It was shown that the TG lowering effect of garlic may stem from impairment of fatty acid and TG synthesis by water-soluble sulfur compounds. Judging from the maximal inhibition and the IC_{50}, SAC, SEC and SPC are equally potent in inhibiting cholesterol synthesis.
Liu L, Yeh YY. 2001. Orange County Convention Center, Exhibit Hall A4. April 4.	It is indicated that the water-soluble organosulfur compounds of garlic (*S*-allylcysteine, *S*-ethyl cysteine and *S*-propyl cysteine) inhibit cholesterol synthesis by decreasing 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase activity due to posttranslational but not pretranslational modification of HMG-CoA reductase.
Gupta N, Porter T. 2001. J Nutr. 131(6):1662-7.	It was found that the garlic extract, as a whole, selenocysteine, *S*-allyl cysteine, alliin, diallyl trisulfide and diallyl disulfide, substantially inhibited squaline monooxygenase, an enzyme that catalyzes or facilitates the second step in the generation of cholesterol.
Liu L, Yeh YY. 2001. Lipids. 36(4):395-400.	The water-soluble compounds *S*-allylcysteine (SAC) and *S*-propyl cysteine (SPC) could also inhibit triglyceride synthesis. These compounds as well as *S*-ethyl cysteine and γ-glutamyl-*S*-methyl cysteine were also effective at inhibiting fatty acid synthesis. On the contrary, precursor compounds in garlic (alliin, γ-SAC and γ-SPC), were ineffective, at inhibiting fatty acid synthesis, suggesting processing such as aging is necessary for garlic to yield its maximal benefits. SAC and SPC also inhibited the activity of lipogenic or fat-producing enzyme.
Liu L, Yeh YY. 2002. J Nutr. 132(6):1129-34.	It is suggested that *S*-alk(en)yl cysteines inhibit cholesterol synthesis by deactivating 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase through enhanced phosphorylation leaving the levels of messenger RNA (mRNA), or the amount of enzyme, unchanged in cultured hepatocytes. In addition, only *S*-allylcysteine (SAC) appeared to further decrease the activity of HMG-CoA reductase by increasing sulfhydryl oxidation of the enzyme.
Lee Y, Yeh Y-Y. 2003. FASEB J. 17(4):A752. Abst #455.1.	Water-soluble organosulfur compounds in Kyolic: *S*-allylcysteine (SAC), *S*-ethyl cysteine (SEC), *S*-propyl cysteine (SPC) and gamma glutamyl *S*-alk(en)yl cysteines, have shown maximal inhibition on cholesterol synthesis requires a concerted action of these various compounds in human hepatocellular carcinoma (HepG2) cells.
Lin CC, Yin MC. 2007. Br J Nutr. 2007. 99(1):37-43.	The intake of *N*-acetyl cysteine (NAC), *S*-ethyl cysteine (SEC) or *S*-propyl cysteine (SPC) treatment significantly decreased triacylglycerol (TAG) and total cholesterol contents ($P<0.05$) via enhancing the activity and messenger RNA (mRNA) expression of malic enzyme, fatty acid synthase and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase ($P<0.05$).
Malekpour-Dehkordi Z, Javadi E, et al. 2013. Phytother Res. 27(3);357-61.	The human monocyte THP-1 cells were differentiated to macrophage cells in the presence of phorbol 12-myristrate13-acetate (PMA) then treated with different concentrations of *S*-allyl cysteine (SAC) for 24 hours. Results showed that SAC increased the adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1) messenger RNA (mRNA) (1.82-, 2.07- and 2.23-fold) and protein (1.37-, 1.55- and 2.08-fold) expression in macrophage THP-1 cells compared with control (untreated cells). These results suggested that SAC can increase ABCA1 expression in macrophages and may be beneficial in promoting reverse cholesterol efflux.

Circulation-Enhancing/Blood-Thinning

Yokoyama K, Yoshii M, et al. 1988. Oyo Yakuri (Appl Pharmacol). 36(4):301-8.

Kyoleopin® (KLE) and Leopin-5® (LE-5) improved peripheral blood circulation. Specifically, intravenous therapy (i.v.) administration of KLE and LE-5 increased peripheral blood flow in the hind limbs of models immersed in cool water (15°C) for 10 minutes significantly more than control (saline and vehicle). Oral administration of KLE and LE-5 shortened the average time for re-warming in these models. Further, *in vivo* administration of KLE and LE-5 inhibited norepinephrine-induced contraction of an isolated artery demonstrating an α-antagonistic effect) and LE-5 caused relaxation of a depolarized vascular smooth muscle.

Qureshi N, Lin RIS, et al. 1990. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. Aug 28-30, p. 17.

Yu SG, Qureshi N, et al. 1991. National Conference on Cholesterol and High Blood Pressure. Washington, D.C. Apr 8-10.

Abuirmeileh N, Yu SG, et al. 1991. FASEB J. 5(6):A1756. Abst #8048.

AGE and *S*-allylcysteine (SAC) was found to lower levels of plasma thromboxane B_2 and factor 4 (blood clotting factors) by 30% in hypercholesterolemic models. AGE and SAC also decreased platelet aggregation induced by the collagen and adenosine diphosphate, potent clotting agents.

Amagase H, Moriguchi T, et al. 2000. Phytomedicine. 7(2):118.

Allicin and its derived oil-soluble compounds contribute to some of the toxicity found from various forms of garlic, such as anemia and gastrointestinal problems. AGE, on the other hand, prevents hemolysis and loss of flexibility of red blood cells caused by lipid peroxidation. Raw garlic and enteric-coated garlic products (Garlicin, Garlique and Garlinase 4000) deliver allicin directly to the gut causing severe damage to the epithelial mucosa lining of the intestinal tract.

Moriguchi T, Itakasugi N, et al. 2001. J Nutr. 131(3 Suppl):1016S-9S.

AGE significantly prevented the loss of erythrocyte deformability (flexibility of red blood cells to move through small microvessels) caused by lipid peroxidation or oxidative damage. AGE also significantly reduced the generation of thiobarbituric acid reactive substances (TBA-RS) or markers of free radical oxidation, and suppression of intercellular adenosine triphosphate (ATP) caused by lipid peroxidation. Moreover, AGE significantly suppressed not only the hemolysis induced by peroxidation but also hemolysis due to non-peroxidation. These results suggest the possibility that AGE improves microcirculation and rheological blood properties and preserves the structure and function of red blood cells by stabilizing the cell membranes and supporting cell metabolism.

Morihara N, Sumioka I, et al. 2002. Life Sci. 71(5):509-17.

It was observed that AGE increased nitric oxide (NO) production by activating endothelial nitric oxide synthase (eNOS) but not inducible nitric oxide synthase (iNOS). AGE increased NO production roughly 30-40% after administration and returned to basal value after 2 hours.

Morihara N, Sumioka I, et al. 2006. J Nutr. 136(3 Suppl):777S-81S.

The effect of AGE was investigated by administering a single dose of AGE to subjects resulting in a 30-40% increase in nitric oxide (NO) production by activating constitutive NO synthase (cNOS), but not inducible NOS (iNOS). Another experiment found that AGE suppressed the rate of peroxynitrite-induced hemolysis in a dose-dependent manner, which suggests that AGE could be useful for long-term prevention of cardiovascular disease associated with oxidative stress or dysfunctions of NO production.

Al-Qattan KK, Thomson M, et al. 2006. J Nutr. 136(3 Suppl):774S-6S.

Garlic was shown to reduce blood pressure by enhancing the concentration and activity of many vasodilatory agents including nitric oxide (NO).

Harauma A, Moriguchi T. 2006. J Nutr. 136(3 Suppl):769S-73S.

AGE or raw garlic (RW) was administered to hypertensive models for 10 weeks. Both AGE and RW reduced the increase of systolic blood pressure (SBP) compared with the control group after 4 weeks of administration. AGE also showed a decrease of pulse pressure (PP) suggesting blood-vessel extensibility while harmful effects were observed in the RG group, which includes a decrease in erythrocytes, an increase in reticulocytes and generation of polyps were found in the forestomach.

Allison GL, Lowe GM, et al. 2006. J Nutr. 136(3 Suppl):789S-92S.

Repeated experiments of platelet-rich plasma (PRP) in the presence of AGE were found to suppress platelet aggregation and calcium mobilization. Furthermore, the metal-chelating properties of AGE was confirmed when platelets were preincubated with AGE significantly reduced the initial concentration of intracellular calcium.

Smith S, Rahman K, et al. 2010. 3rd International Conference on Hypertension, Lipids, Diabetes and

AGE decreases intraplatelet calcium (Ca^{2+}) and may later two signaling molecules nitric oxide (NO) and cyclic 3'5' guanosine monophosphate (cGMP). Preliminary data using 3-morpholinosydnonimine (sin-1),

Stroke Prevention. Berlin, Germany. Mar 4-6.

a nitric oxide donor, has shown an increase in the inhibition of platelet aggregation when combined with AGE. Sin-1 forms both NO and superoxide peroxynitrite, which provide evidence that AGE may be inhibiting platelet aggregation via NO signaling pathways.

Allison GL, Lowe GM, et al. 2012. Life Sci. 91(25-26):1275-80.

AGE at concentrations in the range of 3.12-12.5% (v/v) inhibited the binding of platelets to fibrinogen by approximately 40% when compared to control values ($P<0.05$). AGE also significantly inhibited the binding of adenosine diphosphate (ADP)-activated platelets to immobilized fibrinogen by 61.5% at 1.56% and 6.25%, respectively ($P<0.05$). AGE was also shown to significantly decrease the amount of first procaspase activating compound (PAC-1) binding to glycoprotein Iib/IIIa (GPIIb/IIIa) by approximately 72% compared with phosphate buffered saline (PBS) control. AGE also increased platelet cyclic adenosine monophosphate (cAMP) ($P<0.01$) levels.

Weiss N, Papatheodorou L, et al. 2013. J Ethnopharmacol. 145(1):162-7.

Endothelial cells were incubated with hypoxanthine, aminopterin, thymidine and methionine (HAT/MET) with and without AGE. HAT/MET incubation significantly increased cellular homocysteine (Hcy) levels without AGE and significantly decreased nitric oxide (NO) output and levels of tetrahydrobiopterin. However, AGE in HAT/MET-treated cells prevented declines in NO output and tetrahydrobiopterin levels, increased cellular levels of cysteine and total glutathione, and prevented glutathione and tetrahydrobiopterin oxidation induced by elevated Hcy, suggesting AGE might be useful in the prevention of endothelial dysfunction.

Rahman K, Lowe GM, et al. 2016. J Nutr. 146(2):410S-415S.

In 14 participants, AGE was shown to decrease platelet aggregation in all of the concentrations tested and the decrease was more marked in the presence of 3-morpholinosyndnonimine (Sin-1), a nitric oxide donor (ranged between 15% and 67%). The presence of a phosphodiesterase inhibitor 3-isobutyl-methylxanthine (IBMX) also led to a decrease of 17-35% in platelet aggregation at all AGE concentrations and a significant decrease in the amounts of cyclic guaninosine monophosphate (cGMP) (24-41%) and cylic adenosine monophosphate (cAMP) (19-70%), respectively, in the presence of 1H-(1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), a soluble guanylyl cylcase (sGC) inhibitor, and 9-(tetrahydro-2-furanyl)-9H-purin-6-amine (SQ22536), an adenylyl cyclase (AC) inhibitor. The presence of AGE significantly inhibited the binding of activated platelets to fibrinogen, preventing changes in platelet shape.

Morihara N, Hino A. 2017. J Nat Med. 71(1):249-56.

AGE was administered to models and was shown to significantly reduce the ability of platelet to aggregate on the 14 day treatment. AGE produced platelets that responded to collagen by significantly increasing the amount of both the extracellular adenosine triphosphate (ATP) and the extra- and intracellular thromboxane B2 (TXB2). AGE treatment also dose-dependently suppressed the phosphorylation of collagen-induced extracellular signal-regulated kinase 2 (ERK), p38 and c-JUN NH_2-terminal kinase (JNK).

Hypotensive/Blood Pressure Lowering Effects

Cruz C, Correa-Rotter R, et al. 2007. Am J Physiol Renal Physiol. 293(5):F1691-8.

AGE was given to nephrectromized models (1.2 ml/kg, i.p.) every other day for 30 days. AGE reduced hypertension suggesting that the antihypertensive effect of AGE is associated with its antioxidant properties and that it may be used to ameliorate hypertension.

Takashima M, Kanamori Y, et al. 2017. Phytomedicine. 24:56-61.

AGE was found to induce the concentration-dependent vasorelaxation of isolated model aortic rings that had been precontracted with norepinephrine for 30 min. AGE's effect was severely impaired in aortic rings lacking endothelium. In addition, the effect of AGE was inhibited by a nitric oxide synthase (NOS) inhibitor and a nitric oxide (NO) scavenger. Moreover, AGE treatment of aorta significantly increased the NO production. The vasorelaxation of aorta was observed only in the presence of L-arginine, a substrate of NOS, when various constituents of AGE were tested.

Matsutomo T, Ushijima M, et al. 2017. J Chromatogr B Analyt Technol Biomed Life Sci. 1046:147-55.

Treatment with AGE (2 g/kg body weight) or S-1-propenylcysteine (S1PC) (6.5 mg/kg body weight; equivalent to AGE 2 g/kg body weight) in spontaneously hypertensive models significantly decreased the systolic blood pressure (SBP) of models after the repeated administration for 10 weeks. After the treatment for 10 weeks, the plasma samples were analyzed and results indicated that 30 endogenous metabolites wer changed by the S1PC treatment. Furthermore, regression analysis showed correlation between SBP and the plasma levels of betaine, tryptophan and 3 LysoPCs.

Takashima M, Kanamori Y, et al. 2017. Phytomedicine. 24:56-61.

In model aortic rings contracted by norepinephrine (NE) for 30 minutes, AGE and other test drugs were added. AGE induced the concentration-dependent vasorelaxation of isolated model aortic rings that had been precontracted with norepinephrine. The effect of AGE was severely impaired in aortic rings lacking endothelium. In addition, the effect of AGE was inhibited by a nitric oxide synthase (NOS) inhibitor and a

nitric oxide (NO) scavenger. Moreover, treatment of aorta significantly increased the NO production. The vasorelaxation of aorta was observed only in the presence of L-arginine, a substrate of NOS.

Reduction of Serum Homocysteine

Yeh Y, Yeh S, et al. 1999. FASEB J. 13(4):A232. Abst #209.12.

Hyperhomocysteinemia is a well-established risk factor for arteriovascular diseases and folate deficiency contributes to this condition. It was found that AGE effectively reduced hyperhomocysteinemia caused by severe folate defiency. When succinylsulfathiazole, an antibiotic that destroys bacteria, was added to an already folate deficient diet, homocysteine (Hcy) levels increased significantly. However, the addition of AGE reduced plasma Hcy by 30% and plasma free homocysteine by 24%.

Amagase H. 2000. Method and Pharmaceutical Composition for Reducing Serum Homocysteine Concentrations. Patent #6,129,918.

Kyolic AGE is patented by the U.S. government for its ability to reduce homocysteine.

Yeh YY, Lim HS, et al 2005. Nutr Res. 25:93-102.

Supplementation with AGE was found to decrease homocysteine levels by 28-33% in models severely deficient in folate.

Yeh YY, Yeh SM. 2006. J Nutr. 136(3 Suppl):745S-9S.

AGE supplementation in hyperhomocysteinemic models (severely induced by folate deficiency) decreased plasma total homocysteine concentration by 30%. Increased *S*-adenosylmethionine and decreased *S*-adenosylhomocysteine concentrations in the liver were also noted. The hypohomocysteinemic effect from AGE is most likely due to impaired remethylation of homocysteine to methionine and enhanced transsulfuration of homocysteine to cystathionine.

Mizuno I, Ushijima M, et al. 2006. Oyo Yakuri (Pharmacometrics). 71(1/2):61-6.

Mizuno I, Sumioka I. 2007. 7th Scientific Meeting of the Japanese Society of Anti-Aging Medicine. Jul 20-21. Abst #P028.

Kyoleopin Neo® (KLEN) orally administered (5 ml/kg body weight) for 8 days (short-term hyperhomocysteinemia) in models down-regulated any increase in plasma homocysteine levels at 1 hour after L-methionine administration ($p<0.05$). For long-term hyperhomocysteinemia, models were subjected to L-methionine feeding (1 to 2 w/w%) for 20 weeks down-regulated homocysteine level by 33-56% in comparison with control ($p<0.05$).

Anti-atherogenic/Anti-atherosclerotic Effects

Lee ES, Steiner M, et al. 1994. Biochem Biophys Acta. 1221(1):73-7.

S-allyl mercaptocysteine (SAMC) and *S*-allyl cysteine (SAC), two constituents in AGE, could inhibit vascular smooth muscle cells (SMC) and umbilical endothelial cell proliferation. SMC proliferation constitutes an essential aspect in the development of atherosclerosis and of restinosis (narrowing or constriction) of blood vessels subjected to angioplasty.

Efendy JL, Simmons DL, et al. 1996. IX International Vascular Biology Meeting. Seattle, WA. Sep 4-8. Abst #090.

Efendy JL, Simmons DL, et al. 1996. In: Proceedings of the 1996 Conference of the Anatomical Society of Australia and New Zealand. Brisbane, Australia, p.19.

Efendy JL, Simmons DL, et al. 1996. J Vasc Res. 33(S1):23. Abst #090.

Efendy JL, Simmons DL, et al. 1997. Atherosclerosis. 132(1):37-42.

The direct effect of AGE on the development of atherosclerosis in hypercholesterolemic conditions was investigated. After being fed a high cholesterol diet in combination with AGE for 8 weeks, models developed 45% fewer fatty streak lesions following angioplasty than those fed a high cholesterol diet alone. AGE significantly reduced neointimal formation or thickening of the artery wall and thoracic aorta fatty streak development (accumulation of fat/cell/tissue on the arterial wall) in hyperlipidemic conditions. Furthermore, AGE inhibited smooth muscle cell proliferation providing protection against the onset of atheroscelerosis.

Ide N, Morihara N, et al. 2000. 3rd International Congress on Phytomedicine. Munich, Germany. Oct 11-13. Phytomedicine. 7(2):49. Abst #SL-100.

AGE was reported that the effects of AGE on vascular homeostasis associated with atherosclerosis using several *in vitro* and *in vivo* systems. AGE inhibited Cu^{2+}-induced low-density lipoproteins (LDL) oxidation dose-dependently. AGE inhibited lactate dehydrogenase (LDH) release indicating cell membrane damage and intracellular glutathione (GSH), as an index of intracellular antioxidant, depletion. The effect of AGE on nitric oxide (NO) production was examined *in vivo* (10 ml/kg, p.o.) suggesting that AGE stimulates constitutive NO synthase inhibitor (cNOS), specifically. AGE inhibits Cu^{2+}-induced LDL oxidation, protects endothelial cells from oxidized LDL (Ox-LDL)-induced injury by preventing intracellular GSH depletion. AGE also modulates NO production *in vivo*, suggesting that AGE may be

useful for the prevention of atherogenesis and thrombin formation.

Campbell JH, Efendy JL, et al. 2001. J Nutr. 131(3 Suppl):1006-S-9S.

AGE significantly reduced the development of atherosclerosis in models with high cholesterol. After being fed a high cholesterol diet in combination with AGE (0.8mg/kg body weight/day) or alone, AGE significantly reduced the percent surface area covered by fatty streaks (25 ± 3% vs 70 ± 8%) and reduced aortic arch cholesterol (1.7 ± 0.2 vs 2.1 ± 0.1 mg/chol/g tissue). AGE significantly inhibited the development of thickened, lipid-filled lesions in preformed neointimas produced by a balloon catheter injury of the right carotid artery in cholesterol-fed models (23.8 ± 2.3% vs 42.6 ± 6.5%). AGE completely prevented vascular smooth muscle phenotypic change from the contractile, high volume fraction of myofilament ($V_v$myo) state in test in an *in vitro* model, and potentially inhibited smooth muscle cell proliferation with a median effective dose (ED_{50}) of 0.01%. AGE also inhibited the accumulation of lipid in cultured macrophages and smooth muscle.

Ide N, Keller C, et al. 2006. J Nutr. 136(3 Suppl):755S-8S.

Weiss N, Abahji T, et al. 2006. XIV International Symposium on Atherosclerosis. Rome, Italy. Jun 18-22. Atherosclerosis. 7:Abst #Tu-P10:143.

Coincubation of human monocytes/macrophages (THP-1) with AGE inhibited homocysteine (Hcy)-induced CD36 expression by 61.8 ± 13.9%, compared with control conditions. AGE also slightly inhibited oxidized low-density lipoproteins (Ox-LDL) uptake into THP-1 cells by 85.6 ± 2.8% of control conditions, which suggest that AGE could modulate the formation of early atherosclerotic lesions.

Ide N, Morihara N, et al. 2007. Planta Medica: 55th International Congress and Annual Meeting for Medicinal Plant Research. Graz, Austria. Sep 2-6. 73:967.

Papatheodorou L, Morihara N, et al. 2007. 55th International Congress and Annual Meeting for Medicinal Plant Research. Graz, Austria. Sep 2-6. 73:966-7. Abst #P456.

Morihara N, Papatheodorou L, et al. 2009. XV International Symposium on Atherosclerosis. Boston, MA. Jun 14-18.

Human endothelial cells incubated with hypoxanthine, aminopterin, thymidine and methionine (HAT/MET) with and without AGE (5 mg/mL) slightly increased tetrahydrobiopterin (BH4) in control cells (5.33 ± 0.65 pmol/mg) and prevented the decline in BH4 in HAT/MET treated cells (5.23 ± 0.71 pmol/mg). AGE increased cellular levels of total thiols and glutathione and prevented HAT/MET-induced decrease in endothelial nitric oxide (NO) release. Based on these results, AGE maintains NO bioavailability in endothelial cells even under conditions of elevated homocysteine (Hcy) levels via increasing cellular BH4 levels, thereby maintaining normal endothelial function.

Chuah SC, Moore PK, et al. 2007. Am J Physiol Heart Circ Physiol. 293(5):H2693-701.

S-allyl cysteine (SAC) significantly lowered mortality and reduced infarct size of acute myocardial infarction (AMI) in models. SAC also increased left ventricular cystathionine-γ-lyase (CSE) activity, which is the enzyme responsible for hydrogen sulfide (H_2S) production in the heart ($P<0.01$), thus, also had higher plasma H_2S concentration compared with controls and the SAC + propagylglycine (PAG)-treated group. Protein expression studies revealed that SAC upregulated CSE expression (1.1-fold of control; $P<0.005$). This study provides novel evidence that SAC is protective in myocardial infarction via a H_2S-related pathway.

Padmanabhan M, Rajadurai M, et al. 2008. Basic Clin Pharmacol Toxicol. 103(6):507-13.

Models were pre-treated with *S*-allyl cysteine (SAC) (50, 100 and 150 mg/kg) daily for a period of 45 days then subcutaneously injected with isoproterenol (150 mg/kg) at an interval of 24 hours for 2 days. Pre-treatment with SAC exhibited significant ($P<0.05$) effect and positively altered the biochemical parameters (activities of serum creatine kinase-MB [muscle and brain isozyme], calcium-dependent adenosine triphosphatase and magnesium-dependent adenosine triphosphatase in the heart, serum levels of iron and uric acid, levels of plasma iron binding capacity, plasma total protein, plasma albumin/globulin ratio and activity of sodium potassium-dependent adenosine triphosphatase in the heart, levels of glycoproteins in the serum and heart). From this study, SAC showed a protective role in isoproterenol-induced myocardial infarction in models, which may be due to free radical scavenging, antioxidant and membrane stabilizing properties.

Morihara N, Ide N, et al. 2009. XV International Symposium on Atherosclerosis. Boston, MA. Jun 14-18.

Morihara N, Ide N, et al. 2010. Phytother Res. 24(4):602-8.

AGE dose-dependently and significantly suppressed cluster of differentiation 36 (CD36) expression by up to 60% in both phorbol 12-myristate 13-acetate (PMA)-stimulated human acute monocytic leukemia cell line (THP-1) and primary human monocytes incubated with and without troglitazone. AGE also significantly inhibited 1,1'-dioctadecyl-3,3,3',3'-tetra-methylindocyanide perchlorate (DiI)-labeled oxidized low-density lipoproteins (DiI-OxLDL) uptake into PMA-stimulated THP-1 cells, inhibited CD36 expression induced by the peroxisome proliferator receptor activator γ (PPARγ) agonist troglitazone, decreased the binding of nuclear proteins to a consensus peroxisome proliferator gamma response element (PPRE) sequence compared to control and inhibited cluster of differentiation 11b (CD11b) together with CD36 expression. These findings suggest that AGE inhibits differentiation of cultured monocytes into

macrophages presumably via modulation of the PPARγ pathway, which may partly explain the antiatherosclerotic properties of AGE.

Weiss N, Morihara N, et al. 2009. 7th International Conference on Homcysteine Metabolism. Prague, Czech Republic. Jun 21-25.

In cultured human endothelial cells (EC), cellular levels of homcysteine (Hcy) were increased by methionine supplementation and inhibition of folate metabolism using hypoxanthine, aminopterin and thymidine (H/M). Cellular thiol redox equivalanets were increased by incubation with AGE. AGE increased nitric oxide (NO) release in control cells and prevented H/M induced decreased in NO output, increased tetrahydrobiopterin (THB) levels in both native and in H/M cells. AGE also significantly increased cysteine levels by 2 to 2.5 fold and total glutathione levels by 1.5 to 1.8 fold compared to control and H/M, and prevented glutathione oxidation as indicated by a glutathione disulfide (GSSG)/glutathione (GSH) ratio comparable to control conditions. This data shows that AGE prevents glutathione oxidation, decreases THB levels and preserves NO output from EC even under conditions of elevated Hcy levels.

Morihara N, Ide N, et al. 2011. J Ethnopharmacol. 134(3):711-6.

Coincubation with AGE (5 mg/ml) significantly suppressed cluster of differentiation 36 (CD36) expression in human monocytes (THP-1) derived macrophages by 48.6 ± 9.0% compared to homocysteine (Hcy)-incubated cells only. AGE (1-5 mg/ml) dose-dependently inhibited Hcy-induced CD36 expressionin primary human macrophages, and decreased binding of nuclear proteins to a peroxisome proliferator receptor activator γ (PPARγ) response element. Preincubation with AGE significantly inhibited 1,1'-dioctadecyl-3,3,3',3'-tetra-methylindocyanide perchlorate (DiI)-labeled oxidized low-density lipoprotein (OxLDL) uptake.

Malekpour-Dehkordi Z, Javadi E, et al. 2013. Phytother Res. 27(3):357-61.

ATP-binding cassette transporter A1 (ABCA1) is a key mediator of cholesterol efflux to apoA-I in lipid loaded macrophages, which is the first step of reverse cholesterol transport *in vivo* and is critical in preventing atherosclerosis. Human monocyte THP-1 cells were differentiated to macrophage cells were then treated with different concentrations (10, 20 and 40 mM) of *S*-allyl cysteine (SAC) for 24 hours. Results showed that SAC increased ABCA1 mRNA (1.82-, 2.07- and 2.23-fold) and protein (1.37-, 1.55- and 2.08-fold) expression in macrophage THP-1 cells compared with control (untreated cells).

Yamakawa T, Matsumoto T, et al. 2014. Food Nutr Sci. 5:177-84.

It was found that primary human coronary artery smooth muscle cells (HCASMC) showed the increase alkaline phosphatase (ALP) activity, when cultured with ascorbic acid, β-glycerophosphate, dexamethasone (IM), and supplemented with conditioned medium from macrophages (MCM). Then the effect of AGE subdivided fractions and several compounds found in AGE was tested, and it was found that ((+)-(2S,3R)-dehydrodiconiferyl alcohol, a dilignol compound existed in hydrophobic fraction of AGE, inhibited ALP activity in HCASMC.

Morihara N, Hino A, et al. 2016. J Nutr. 146(2):460S-463S.

To clarify the mechanism of AGE's action to retard atherosclerosis, models were fed a standard diet with or without 3% AGE for 12 or 24 weeks. The atherosclerotic lesion areas in the aortas of the models were 87 and 114 times as great ($P < 0.01$) as those in control models at 12 and 24 weeks, respectively. AGE significantly inhibited the progression of atherosclerotic lesion area in models by 22% ($P < 0.05$) at 12 weeks. Serum concentrations of total cholesterol (TC) and triglycerides (TGs) were also shown to be significantly higher than those in control models at 12 and 24 weeks. AGE treatment significantly suppressed the increases in serum concentrations of TC and TGs by 21% ($P < 0.05$) and 19% ($P < 0.05$) at 24 weeks, respectively, and reduced the relative abundance of monocytes plus macrophages ($CD11b^+$ cells) in models by 24% ($P < 0.05$) at 12 weeks. This suggests that AGE's antiatherosclerotic activity is at least partly due to the suppression of inflammation and lipid deposition in the vessels during the early stage of atherosclerotic development in models.

Morihara N, Hino A, et al. 2017. Mol Nutr Food Res. 61(10). Doi: 10.1002/mnfr.201700308.

Apolipoprotein E-knockout (ApoE-KO) models were fed a standard diet with or without 3% AGE for 12 weeks. AGE inhibited the progression of atherosclerotic lesion by 27% and reduced the level of C-reactive protein (CRP) and thromboxane B_2 (TXB_2) in serum by 39 and 33%, respectively, compared to ApoE-KNO models without AGE treatment. AGE also decreased the level of tumor necrosis factor alpha (TNF-α) in the liver by 35%, decreased interleukin-1 receptor-associated kinase 4 (IRAK4) by 60% and almost doubled the level of mmune r-AMP-activated protein kinase (p-AMPK) in the liver.

Sickle Cell Anemia

Ohnishi ST, Ohnishi T, et al. 2000. Nutrition. 16(5):330-8.

A certain population of red blood cells in patients with sickle cell anemia has elevated density and possesses an abnormal membrane called dense cells, which have a tendency to adhere to neutrophils, platelets, and vascular endothelial cells, thus, triggering vasoocclusion. AGE inhibited the formation of dense cells by 50% *in vitro* at a concentration of 4 mg/ml. If orally taken, AGE could reduce dense cell

formation, which may be beneficial for sickle cell anemia.

Ohnishi ST, Ohnishi T, et al. 2001. Blood Cells Mol Dis. 27(1):148-57.

AGE effectively inhibited *in vitro* dehydration of sickle red blood cells induced by potassium-chloride cotransport (K-Cl) or red cell storage. At 6 mg/ml of AGE, dehydration of sickle red blood cells was inhibited to 30% of the control level. Chloride efflux measurements demonstrated that AGE effectively inhibited anion transport in red blood cells.

Metabolic Syndrome

Perez-Torres I, Torres-Narvaez JC, et al. 2016. Molecules. 21(11). Pii:E1425.

The antioxidant effects of AGE on models with metabolic syndrome (MS) were studied in 4 groups: control models plus saline solution (C + SS), MS models plus saline solution (MS + SS), control models receiving AGE (C + AGE 125 mg/kg/12 h) and MS models with AGE (MS + AGE). MS + SS had increased triglycerides, systolic blood pressure, insulin, leptin, HOMA index and advanced glycation end products, which AGE returned their levels to control values ($p<0.01$). AGE decreased cholesterol ($p = 0.05$), and had the opposite effects of those in the MS + SS group by increasing glutathione and GPx activity ($p=0.05$) and reducing lipid peroxidation ($p=0.001$). AGE also had opposite effects of MS group by improving vascular functioning ($p=0.001$), decreasing coronary vascular resistance ($p=0.001$), increasing cardiac performance, and increasing NO measured in the perfusate liquid from the heart and serum citrulline, nitrites/nitrates ($p<0.01$).

Diabetes

Ahmad MS, Ahmed N. 2006. J Nutr. 136(3 Suppl):796S-9S.

Ahmad MS, Pischetsrieder M, et al. 2007. Eur J Pharmacol. 562(1-3):32-8.

Elosta A, Slevin A, et al. 2007. 9th International Symposium on Maillard Reaction. The Maillard Reaction. Munich, Germany. Sep 1-5, p. 161. Poster # BM7.

S-allyl cysteine (SAC), the key component in AGE, was shown to inhibit advanced glycation endproduct (AGEP) formation *in vitro*, which can help to prevent diabetic complications.

Smith S, Rahman K, et al. 2010. International Conference on Hypertension, Lipids, Diabetes and Stroke Prevention. Berlin, Germany. Mar 4-6, p. 231.

The effect of AGE was studied using two models of spontaneous diabetes, TSOD and ddY-H. TSOD models fed a diet containing 4% (w/w) AGE for 6 weeks had a lower levels of blood glucose compared to control and glucose intolerance was ameliorated. The level of sterol regulatory element-binding protein 1c (SREBP1c), peroxisome proliferator receptor activator γ (PPAR-γ) and glucose transporter 2 (GLUT2) messenger RNAs (mRNAs) was lower in the liver of AGE-fed TSOD models. Similar results were obtained by experiments using the other diabetic model ddY-H. The data indicated that AGE prevents occurrence of glucose intolerance and fatty liver in diabetic models, suggesting that AGE is useful for the prevention and treatment of type 2 diabetes.

Saravanan G, Ponmurugan P. 2010. Plant Food Hum Nutr. 65(4):374-8.

The possible protective effects of *S*-allyl cysteine (SAC) on the antioxidant defense system of pancreas in streptozotocin (STZ)-induced diabetes in models were evaluated. The levels of glucose, thiobarbituric acid reactive substances (TBARS), and enzymatic antioxidants reverted back to near control levels after treatment with SAC. These findings suggest that SAC treatment exerts a therapeutic protective nature in diabetes by decreasing oxidative stress.

Saravanan G, Ponmurugan P, et al. 2010. Phytomedicine. 17(14):1086-9.

S-allyl cysteine (SAC) was administered orally for 45 days to normal and streptozotocin-induced (STZ) diabetic models. STZ-induced diabetic models showed significant increase in blood glucose and glycoprotein components such as hexose, hexosamine, ructose and sialic acid in plasma, liver and kidneys of diabetic models. SAC administration normalized all the above-mentioned biochemical parameters. This study indicates that SAC possesses a significantly beneficial effect on the glycoprotein moiety in addition to its antidiabetic effect.

Saravanan G, Ponmurugan P. 2011. Exp Toxicol Pathol. 64(6):639-44.

Oral administration of *S*-allyl cysteine (SAC) at a dose of 150 mg/kg bodyweight per day to streptozotocin (STZ)-induced diabetic models for a period of 45 days resulted in a significant reduction in fasting blood glucose, cholesterol (TC), triglycerides (TG), free fatty acids, phospholipids, low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and elevation of high-density lipoprotein cholesterol (HDL-C) in comparison with diabetic control group. SAC administration to diabetic models also decreased the concentrations of fatty acids, namely palmitic, stearic (16:1) and oleic acid (18:1), whereas linolenic (18:3) and arachidonic acid (20:4) were elevated. The results indicate that SAC showed an antihyperlipidemic effect in addition to its antidiabetic effect in experimental diabetes.

Saravanan G, Ponmurugan P. 2011. Chem Biol Interact. 189(1-2):100-6.

S-allyl cysteine (SAC) was administered orally for 45 days to control and streptozotocin (STZ)-induced diabetic models. SAC administration to models showed a decrease in plasma glucose, thiobarbituric acid reactive substances (TBARS), hydroperoxide and oxidized glutathione (GSSG). In addition, the levels of plasma insulin, superoxide dismutase, catalase, glutathione peroxidase (GPx) and reduced glutathione (GSH) were increased in SAC treated diabetic models, which these findings were supported by histological observations of the liver and kidney. This indicates that SAC possesses a significant favorable effect on antioxidant defense system in addition to its antidiabetic effect.

Saravanan G, Ponmurugan P, et al. 2012. J Trace Elem Med Biol. [Epub ahead of print].

In streptozotocin (STZ)-induced diabetic models, *S*-allyl cysteine (SAC) was administered orally for 45 days. The levels of glucose, iron, ferritin, bilirubin and heart heme oxygenase activity (HO) in liver were decreased whereas levels of insulin, ransferring and δ-aminolevulinic acid dehydratase activity (δ-ALA-D) in tissues increased in SAC treated diabetic models. These findings suggest that SAC could have a protective effect against alterations in oxidative stress induced iron metabolism in the diabetic state.

Saravanan G, Ponmurugan P. 2012. J Diabetes Complications. 26(4):280-5.

S-allyl cysteine (SAC) was administered orally for 45 days to control and streptozotocin (STZ)-induced diabetic models. SAC administration to diabetic models showed a decrease in plasma glucose, thiobarbituric acid reactive substances (TBARS), hydroperoxide and glycated hemoglobin (HbA1C). In addition, the levels of plasma insulin, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), total protein, albumin, thyroid hormone and thyroid-stimulating hormone (TSH) were increased in SAC-treated diabetic models. From these findings, SAC might be acting through activation in the synthesis and/or secretion of circulating thyroid hormones which in turn stimulate the synthesis of insulin.

Shiju TM, Rajesh NG, et al. 2013. Indian J Pharmacol. 45(1):18-23.

AGE supplemented at a dose of 500 mg/kg body weight/day for 12 weeks to streptozotocin-induced diabetic models showed a significant change in urine ($P<0.001$) and serum ($P<0.01$) albumin, creatinine, urea nitrogen and glycated hemoglobin. Serum lipid profile of the diabetic models were altered significantly ($P<0.05$) compared to that of the control models. However, the diabetic models supplemented with AGE restored all of these biochemical changes.

Naidu PB, Sathibadu Uddandrao VV, et al. 2016. Can J Diabetes. 40(5):442-8.

Diabetes was induced in models by streptozotocin (STZ) and nicotinamide (NA). S-allylcysteine (SAC) administration in models with diabetes showed effects similar to those of gliclazide in decreasing blood glucose, adlose reductase (AR), sorbitol dehydrogenase (SDH), sorbitol, fructose, glycosylated hemoglobin, thiobarbituric acid-reactive substances (TBARS) and hydroperoxides levels and significant increases in insulin, hemoglobin and glutathione (GSH) activity. Histopathologic studies also revealed the protective effect of SAC on pancreatic beta cells.

Miki S, Inokuma KI, et al. 2017. Mol Nutr Food Res. 61(5). Doi: 10.1002/mnfr.201600797.

Tsumura Suzuki Obese-Diabetes (TSOD) models were fed a standard diet with or without AGE for 19 weeks. AGE treatment lowered the blood glucose level and significantly reduced the plasma level of glycated albumin in TSOD models as compared with those without AGE treatment. In addition, AGE treatment increased the level of phosphorylated AMP-activated protein kinase (AMPK) in the adipose tissue, liver and muscle that played an important role in the maintenance of insulin sensitivity. Moreover, AGE treatment also suppressed the mRNA expression of fatty acid synthase and monocyte chemoattractant protein 1, one of the representative inflammatory chemokines, in the adipose tissue but not in the liver.

Elosta A, Slevin M, et al. 2017. Sci Rep. 7:39613.

Proteins were glycated by incubation with sugars (glucose, methylglyoxal or ribose) ± 5-15 mg/mL of aged and fresh garlic extracts. Aged garlic inhibited advanced glycation endproducts (AGEs) by 56.4% compared to 33.5% for an equivalent concentration of fresh garlic extract. Similarly, aged garlic had a higher total phenolic content (129 ± 1.8 mg/g) compared to fresh garlic extract (59 ± 1.2 mg/g).

Baluchnejadmojarad T, Kiasalari Z, et al. 2017. Eur J Pharmacol. 794:69-76.

Streptozotocin (STZ)-diabetic models treated with *S*-allylcysteine (SAC) (150 mg/kg) for 7 weeks ameliorated cognitive deficits through modulation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2)/(nuclear factor-kappa B (NF-κB)/toll-like receptor 4 (TLR4)/heme oxygenase 1 (HO-1), and acetylcholinesterase and attenuation of associated oxidative stress and neuroinflammation.

Cardioprotection with Atenolol during Cardiac Toxicity

Avula PR, Asdaq SM, et al. 2014. Indian J Pharmacol. 46(1):94-9.

Models were administered AGE at two different doses of 2 ml/kg or 5 ml/kg orally, whereas *S*-allylcysteine (SAC) was administered either at a dose of 13.1 mg/kg or 32.76 mg/kg, given alone or in combination with atenolol, every alternate day for 3 weeks. Two doses of isoproterenol were administered

to models at the end of treatment. AGE and SAC administration caused a decrease in serum lactate dehydrogenase (LDH) and creatinine kinase-MB (CK-MB) activities and an elevation of LDH and CK-MB activities in heart tissue homogenate (HTH). Atenolol alone or in combination with AGE and SAC demonstrated similar changes in biomarker activities. AGE showed dose-dependent cardioprotection while SAC with atenolol combated more effectively the myocardial dysfunction during isoproterenol induced cardiotoxicity in models.

Thomson M, Al-Qattan KK, et al. 2016. BMC Complement Altern Med. 16:17.

Streptozotocin (STZ)-induced diabetic models were divided into two groups: control diabetic group (CD) and AGE-treated diabetic group (AGE-D). The AGE-D was divided into 3 groups according to AGE treatment i.p. at 100, 300 and 600 mg/kg daily for 8 weeks. A control normal group (CN) was also included for reference. CD group showed significant loss of body weight (over 50%) and decreased serum insulin concentration (10 fold) and total anti-oxidant level and catalase activity (45-70%) in serum, kidney and liver compared to the CN group. Conversely, the CD models had an elevated blood glucose (nearly 4 fold), serum, cholesterol (nearly 2 fold) and triglycerides (>2 fold), erythrocyte glycated hemoglobin (Ghb, 3 fold) and kidney and liver lipid peroxidation (MDA levels). AGE treatment positively reversed the diabetic changes in the targeted parameters to levels significantly lower than those measured in the CD group and the degrees of attenuation were almost dose dependent especially with the two higher doses.

Cardiovascular Review

Rahman K. 2007. Mol Nutr Food Res. 51(11):1335-44.

Garlic, including AGE, is reported to prevent cardiovascular disease by multiple effects, one of which is the inhibition of platelet aggregation. *In vitro* studies indicate that garlic prevents inhibition of platelet aggregation by inhibiting cyclooxgenase activity and thus thromboxane A2 formation, by suppressing mobilization of intraplatelet calcium (Ca^{2+}), and by increasing levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) and also activates nitric oxide synthase (NOS), leading to an increase in platelet-derived NO. It can also interact directly with the fibrinogen receptor glycoprotein Iib/IIIa (GPIIb/IIIa), thus reducing the ability of platelets to bind to fibrinogen.

Gorinstein S, Jastrzebski Z, et al. 2007. Mol Nutr Food Res. 51(11):1365-81.

The contemporary data concerning atherosclerosis and protecting properties of garlic, which includes AGE, is reviewed. Numerous *in vitro* studies have confirmed the ability of garlic including AGE, to reduce the parameters of the risk of atherosclerosis: total cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, oxidixed LDL (Ox-LDL). *In vivo* studies were reviewed on with garlic including AGE, and cholesterol supplemented diets. The positive influences of garlic, including AGE, on plasma lipids, proteins, antioxidant activity and some indices of blood coagulation are dose-dependent. Garlic, including AGE, could be a valuable component of atherosclerosis-preventing diets only in optimal doses. Many recently published reports show that garlic, including AGE, possesses plasma lipid-lowering and plasma anticoagulant and antioxidant properties and improves impaired endothelial function.

Charron CS, Dawson HD, et al. 2016. J Nutr. 146(2):444S-449S.

The determination of how garlic influences mRNA gene expression has proven valuable to understanding the mechanisms of garlic bioactivity. However, preclinical studies that investigate the benefits of garlic far outnumber human studies and have made frequent use of mRNA gene expression measurement. The There is an immediate need to understand mRNA gene expression in humans as well. This paper reviews how garlic influences gene expression *in vivo* and *in vitro*.

Bradley JM, Organ CL, et al. 2016. J Nutr. 146(2):403S-409S.

This review discusses the current knowledge concerning the cardioprotective effects of garlic-derived diallyl polysulfides. There is emerging evidence that hydrogen sulfide (H_2S) has cardioprotective and cytoprotective properties in recent years. Allicin, the active metabolite in garlic, is readily degraded into organic diallyl polysulfides that are potent H_2S donors in the presence of thiols. The enhancement of endogenous H_2S has an impact on vascular reactivity shown in preclinical studies, and the administration of H_2S prevents myocardial injury and dysfunction in cardiovascular disease (CVD) models. It is hypothesized that these beneficial effects of garlic may be mediated by H_2S-dependent mechanisms.

Tsuneyoshi T. 2020. Exp Ther Med. 19(2):1500-3.

In this review, a current overview of the antioxidant effects of AGE and sulfur-containing amuno acids *S*-1-propenylcysteine (S1PC) and *S*-allylcysteine (SAC) are discussed. S1PC has the unique prpperty of downregulating BTB domain and CNC homolog 1 (BACH1), a transcriptional repressor that competes with nuclear factor erythroid 1 like 2 (NRF2), in a nitric oxide (NO)-dependent manner and enhancing the expression of antioxidant genes reciprocally regulated by NRF2 and BACH1.

Miki S, Suzuki JI, et al. 2020. Exp Ther Med. 19(2):1462-7.

AMP-activated protein kinase (AMPK) is ubiquitously expressed serine/threonine kinase and an important regulator of energy metabolism. The decreased activity of AMPK induced by low-grade chronic inflammation has been implicated in several diseases, including type 2 diabetes and atherosclerosis.

However, the activation of AMPK by natural and synthetic products can ameliorate these diseases through the inhibition of inflammation. The mechanisms through which AGE activates AMPK, as well as the mechanisms through which the activation of AMPK by AGE modulates the inflammatory response in disease models.

Liver Protective/Detoxification Effects

Protected Cells from Toxic Carbon Tetrachloride and Paracetamol (Acetaminophen)

Nagai K, Yamawaki M. 1974. Yakuri to Chiryo (Jpn J Pharmacol Ther). 2(10):1622-35.

The protective effects of Kyoleopin® (KLE) were studied on liver tissue damage induced by inhalation of carbon tetrachloride (CCl_4). At dosages of both 0.2 and 2.0 ml for 10 days preceding lung inhalation of CCl_4, KLE treated models maintained normal healthy liver tissue, as evidenced by various parameters of liver tissue analysis, whereas control models exposed to CCl_4 alone experienced significant liver damage.

Nakagawa S, Yoshida S, et al. 1985. Hiroshima J Med Sci. 34(3):303-9.

AGE and its constituents, *S*-allyl cysteine (SAC), *S*-allyl mercaptocysteine (SAMC) and *S*-propyl cysteine (SPC), completely suppressed the cytotoxicity of the potent liver toxin carbon tetrachloride (CCl_4), whereas 4 positive controls drugs (vitamin E, piperonyl butoxide, glycyrrhizin and glutathione) were found to be less effective at protecting liver cells.

Nakagawa S, Kasuga S, et al. 1988. Phytother Res. 1(0):1-4.

AGE and its constituents, *S*-allyl cysteine (SAC) and *S*-allyl mercaptocysteine (SAMC), protected the liver cells from the liver toxins paracetamol (acetaminophen) and carbon tetrachloride (CCl_4) that induce acute hepatitis. Both SAC and SAMC appeared to enhance the activity of glutathione, a detoxifying enzyme, and acted as chemical scavengers. These garlic constituents were found to be more effective than the other chemicals used.

Sumioka I, Matsuura T, et al. 1998. Jpn J Pharmacol. 78(2):199-207.

S-allyl mercaptocysteine (SAMC) at 100 mg/kg, p.o., given to models 2 and 24 hours before administration of acetaminophen (APAP; 500 mg/kg, p.o.) prevented liver damage as shown by a reduction in alanine aminotransferase (ALT) activity, which is enhanced by APAP. ALT was shown to decrease by 79%, 97% and 100% when APAP was given in conjunction with 50, 100 and 200 mg/kg of SAMC. SAMC also prevented the reduction in glutathione induced by APAP administration. One mechanism proposed for liver protection was inhibition of cytochrome P450 2E1 (CYP2E1) activity since SAMC suppressed an enzyme representative of P450 2E1 activity. CYP2E1 is a major enzyme responsible in bioactivation of APAP. SAMC pretreatment also suppressed the increase in hepatic lipid peroxidation and the decrease in hepatic Coenzyme Q9 (CoQ_9H_2) suggesting an antioxidative effect.

Amagase H, Matsuura H, et al. 2000. Ch. 6. In: Phytochemicals and Phytopharmaceuticals. AOCS Press. Champaign, IL, pp. 62-78.

Oral consumption of AGE before carbon tetrachloride (CCl_4) injection significantly reduced the amount of pentane in the breath by 80% compared to control models showing a suppression of lipid peroxide formation.

Sumioka I, Matsuura T, et al. 2001. Eur J Pharmacol. 433(2-3):177-85.

S-allyl mercaptocysteine (SAMC), a constituent in AGE, could ameliorate the toxicity of acetaminophen-induced liver damage by reducing liver cell death and mortality (43% to 0%). It is suggested that SAMC might be useful as an antidote for acetaminophen overdose.

Hsu CC, Lin CC, et al. 2006. Food Chem Toxicol. 44(3):393-7.

Acetaminophen-induced depletion of glutathione (GSH) content in blood and organs could be lessened by *S*-allyl cysteine (SAC) and *S*-propyl cysteine due to their antioxidant tendencies. Consequently, models demonstrated suppressed oxidation, inflammation and coagulation with improved liver function.

Kodai S, Takemura S, et al. 2007. Free Radic Res. 41(4):489-97.

S-allyl cysteine (SAC) administered intraperitoneally (50-200 mg/kg) to models and was found to significantly suppress the increases of plasma alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) levels and hepatic total level of hydroxyoctadecadienoic acid (HODE), a new oxidative stress biomarker correlated with the amount of liver damage. SAC dose-dependently attenuated lipid peroxidation and increases in plasma malondialdehyde and hepatic 4-hydroxy-2-nonenal levels induced by carbon tetrachloride (CCl_4).

Xiao J, Liong EC, et al. 2012. Eur J Nutr. 5(3):323-33.

Models were intraperitoneally injected with carbon tetrachloride (CCl_4) to induce acute hepatotoxicity with or without a 2-hour pre-treatment of *S*-allylmercaptocysteine (SAMC) intraperitoneal injection. After 8 hours, SAMC was shown to reduce CCl_4-triggered cellular necrosis and inflammation in the liver under histological analysis. Since co-treatment of SAMC and CCl_4 enhanced the expressions of antioxidant enzymes, reduced the nitric oxide (NO)-dependent oxidative stress and inhibited lipid peroxidation induced by CCl_4. SAMC also ameliorated hepatic inflammation induced by CCl_4 via inhibiting the activity of nuclear factor κ B (NF κB) subunits p50 and p65, thus reducing the expressions of pro-inflammatory cytokines, mediators and chemokines, as well as pro-regenerative factors at both transcriptional and translational levels.

Enhanced Metabolism of Acetaminophen in Humans

Gwilt P, Lear CL, et al. 1994. Cancer Epidemiol Biomarkers Prev. 3(2):155-60.

AGE enhanced the metabolism or detoxification of the pain killer acetaminophen (1 g), as shown by increased sulfate conjugation and glucuronide formation of this drug for excretion from the kidneys, in 16 subjects taking 10 mL of AGE Liquid daily for 3 months. Since the metabolism of acetaminophen is very similar to that of carcinogens and the effects of AGE were only slight, the results suggest that AGE inhibits carcinogenesis through a mechanism other than modification of drug metabolism. Potential theories include the fact that AGE increased glucuronidation, and some evidence of enhanced sulfate conjugation may explain another mechanism.

Prevented the Mutagenic Effects of the Liver Toxin Aflatoxin B_1

Tadi PP, Teel RW, et al. 1990. Int Clin Nutr Rev. 10:423-9.

Tadi PP, Teel RW, et al. 1991. Nutr Cancer. 15(2):87-95.

AGE was found to protect liver tissue from damage caused by aflatoxin B_1 (AFB_1). Specifically, it inhibited binding of AFB_1 to DNA and mutation of liver cells. AGE also significantly decreased toxic metabolites of AFB_1 and increased nontoxic metabolites, such as glucuronide and glutathione.

Prevented the Toxic Effects of Phenobarbital and Bromobenzene

Wang BH, Zuzel KA, et al. 1998. Toxiciology. 126(3):213-22.

AGE protected liver cells from mmune rbital and bromobenzene exposure. Phenobarbital is a sedative drug that exacerbates the destructive effects of bromobenzene, an industrial solvent. Together, mmune rbital and bromobenzene elicit the generation of a potent liver toxin bromobenzene-3,4-oxide, that causes liver damage *in vitro*. This model is used to assess liver damage and/or potential chemicals or drugs that may be liver protective. AGE at a concentration of 1-5% (v/v) reduced the toxicity of bromobenzene in a concentration-dependent manner as judged by all of the parameters of viability studied. Lipid peroxidation, on the other hand, was reduced to control levels even at the lowest concentration of AGE. The mechanism appeared to be sparing of reduced glutathione by AGE.

Wang BH, Zuzel K, et al. 1999. Toxicology. 132(2-3):215-25.

Precision-cut liver slices from mmune rbital-induced models were incubated for 6 hours with the model liver toxin bromobenzene (BB). Severe toxicity was noted by decreased potassium, adenosine triphosphate and the antioxidant glutathione, release of lactate dehydrogenase into the medium and increased the marker of oxidation thiobarbituric acid reactive substances (TBA-RS). Pretreatment of models for 7 days with AGE at doses of 2 or 10 ml/kg a day dramatically reduced BB toxicity with the larger dose being even more effective. The mechanism involves both an elevation (up to 80%) of the glutathione (GSH) content of the liver seen only after pretreatment with AGE and a GSH-sparing effect by a constituent of AGE. Since similar protective effects against BB toxicity were seen when up to 1 mM *S*-allyl cysteine (SAC) was added to the culture medium of liver slices, SAC or a metabolite may be the GSH-sparing agent.

Protected the Hepatotoxic Effects of p-Dimethylaminoazobenzene and Phenobarbital

Pathak S, Catanzaro R, et al. 2018. Drug Chem Toxicol. 12:1-14.

Two liver carcinogens, p-dimethylaminoazobenzene and mmune rbital, were chronically fed to models to produce hepatotoxicity. Compared to the controls, remarkable elevation in the activities of lactate dehydrogenase, gamma glutamyl transferase and decline in catalase and glucose-6-phosphate dehydrogenase were observed. Daily administration of AGE could favorably modulate the elevated levels of toxicity biomarkers serum triglyceride, creatinine, urea, bilirubin, blood urea nitrogen except total cholesterol. It also altered the levels of blood glucose, high-density lipoprotein (HDL)-cholesterol, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and hemoglobin contents in carcinogen intoxicated models.

Prevention of Arachidonic Acid-induced Liver Injury

Qin XY, Su T, et al. 2020. Exp Ther Med. 19(2):1522-7.

The potential role of AGE in preventing the growth-suppressive effecst of arachidonic acid (AA), an unsaturated fatty acid known to be a pro-inflammatory precursor, on human hepatic cells were examined and we aimed to provide a mechanistic insight regarding the association between the hepatoprotective effects of garlic extract and the inhibition of transglutaminase (TG)2-related crosslinking of nuclear proteins, which is not associated with hepatic lipid partitioning mediated by stearoyl-CoA desaturase-1. We propose that AGE may serve as a therapeutic option for the prevention of chronic liver injury and

inflammation, as well as the prevention of carcinogenesis of fatty livers.

Enhanced Detoxification of Acetaldehyde

Kasuga S, Uda N, et al. 2001. J Nutr. 131(3 Suppl):1080S-4S.

The detoxifying activities of four garlic preparations were investigated including raw garlic juice (RGJ), heated garlic juice (HGJ), dehydrated garlic powder (DGP) and AGE on intoxication by acetaldehyde. HGJ and DGP stimulated acetaldehyde detoxification but RGJ was ineffective. AGE showed significant beneficial effects.

Enhanced Detoxification Enzymes in the Liver

Blakely S, Misio B, et al. 1993. FASEB J. 7:A864. Abst #4990.

AGE enhanced the activity of detoxification and antioxidant enzymes in the liver of models consuming garlic and cholesterol or garlic and high levels of iron. Activity was more pronounced in female models than in male models. Specifically, levels of benzephetamine demethylase, aniline hydroxylase, and glutathione-*S*-transferase were enhanced.

Inhibited Cholestergenesis in HepG2 Cells

Lee Y, Yeh Y-Y. 2003. FASEB J. 17(4):A75. Abst #455.1.

Lee et al. discovered that there is maximal inhibition on cholesterol synthesis when Kyolic AGE (containing *S*-allyl-cysteine [SAC], *S*-ethyl-cysteine [SEC], *S*-propyl-cysteine [SPC] and γ-glutamyl S-alk(en)yl cysteines) is used in addition with various water-soluble sulfur compounds (SAC and/or SPC) in human hepatocellular carcinoma (HepG2) cells.

Anti-Fibrotic Effects

Shinkawa H, Takemura S, et al. 2009. Osaka City Med J. 55(2):61-9.

Hepatic fibrosis was induced in models by porcine serum (PS) intraperitoneal injection. *S*-allyl cysteine (SAC) (0.15% of basal diet) or *N*-acetylcysteine (NAC, 0.45% of basal diet) was orally administered for 12 weeks. SAC and NAC each markedly attenuated the development of hepatic fibrosis and suppressed the PS-induced increase in α-smooth muscle actin (α-SMA) expressions, a marker of hepatic stellate cell (HSC) activation.

Fatty Liver

Xiao J, Ching YP, et al. 2013. Eur J Nutr. 52(1):179-91.

Models fed with a diet with high unsaturated fat (30% fish oil) for 8 weeks developed non-alcoholic fatty liver disease (NAFLD) with or without an intraperitoneal injection of 200 mg/kg *S*-allylmercaptocysteine (SAMC) three times per week. Co-treatment of SAMC attenuated NAFLD-induced liver injury, fat accumulation, collagen formation and free fatty acids (FFAs). SAMC decreased the lipogenesis marker, restored the lipolysis marker, and reduced the expression levels of pro-fibrinogenic factors and diminished liver oxidative stress partly through the inhibition in the activity of cytochrome P450 2E1-dependent pathway. SAMC treatment also partially mitigated NAFLD-induced inflammation via reduction of pro-inflammatory mediators, chemokines and suppressor of cytokine signaling. SAMC also restored altered phosphorylation status of FFAs-dependent MAP kinase pathways and diminished in the nuclear transcription factors (NF-κB and AP-1) activity during NAFLD development.

Takemura S, Minamiyama Y, et al. 2013. J Clin Biochem Nutr. 53(2):94-101.

S-allylcysteine (SAC) was administered to models with nonalcoholic fatty liver disease for 13 weeks. SAC improved hemoglobin A1C, blood glucose, triglyceride, and low-density lipoprotein cholesterol levels. Furthermore, SAC normalized plasma insulin levels. SAC specifically activated the mRNA and protein expression of both peroxisome proliferator-activated receptor α and γ, as well as inhibiting pyruvate dehydrogenase kinase 4 in the liver. Sterol regulatory element-binding protein 1c and forkhead box O1 proteins were also normalized by SAC in the liver.

Chen LY, Chen Q, et al. 2016. Int Immunolpharmacol. 36:23-30.

In a model of alcohol administration and human normal liver cell line cultured with suitable ethanol to mimic the pathological condition of alcoholic fatty liver (AFL), administration of diallyl trisulfide (DATS) significantly lowered the accumulation of intracellular reactive oxygen species (ROS), but antioxidant capacity was increased by DATS. DATS also inhibited heptocyte apoptosis via down-regulating Bax expression and up-regulating Bcl-2 expression, and attenuated alcohol-induced caspase-dependent apoptosis. Lastly, SATS was found to increase the expressions of cystathionine gamma-lyase (CSE) and cystathionine beta-synthase (CBS), the major H2S-producing enzymes.

Maeda T, Miki S, et al. 2019. Exp Ther Med. 18(1):857-66.

Two types of mice: ddY-H mice, an insulin resistance mouse, and ddY-L mice, and normal mice, were

fed an AGE-supplemented diet (4% w/w) for 7 weeks. In the ddY-H mice, the serum levels of glucose and insulin were increased and glucose tolerance was impaired; however, AGE ameliorated these abnormal conditions. AGE did not have these effects in ddY-L mice. Triglyceride (TG) accumulated in the liver and fat absorption from the digestive tract were increased in the ddY-H mice; however, AGE reduced this increase. On the other hand, AGE exerted no such effects in the ddY-L mice. The bacterial composition of the gut microbiota in the feces of ddY-H mice and ddY-L mice was altered in the mice at 9 and 12 weeks of age even when the mice were fed a standard diet. In the ddY-H mice, the relative presence of Lactobacillales was increased, while that of Bifidobacterium, Clostridium cluster XVIII and Prevotella was decreased. The alteration of the bacterial composition in the ddY-H mice was reversed by AGE; however, this effect was not observed in the ddY-L mice. These results indicate that AGE improves abnormal fat accumulation and insulin resistance, and also alters the intestinal flora in ddY-H mice.

Other Liver Protective Effects

Yan SL, Yin MC. 2007. J Food Sci. 72(7):S511-5.

In alcohol-induced acute liver injury in models, preintake of *S*-allyl cysteine (SAC), *S*-ethyl cysteine (SEC), *S*-methyl cysteine (SMC) and *S*-propyl cysteine (SPC) was shown to significantly attenuate subsequent alcohol-induced lipid oxidation, glutathione (GSH) depletion and activity reduction of catalase and glutathione peroxidase ($P<0.05$); also attenuated were the alcohol-induced elevation of C-reactive protein (CRP), interleukin-6 (IL-6), IL-10 and tumor necrosis factor-α (TNF-α) ($P<0.05$) and significantly retarded alcohol-induced cytochrome P450 2E1 (CYP2E1) activity increase ($P<0.05$). In the alleviate study, SAC, SEC, SMC and SPC restored liver GSH content ($P<0.05$); however, only SEC and SPC posttreatments significantly reduced lipid oxidation and alleviated alcohol-induced elevation of CRP, IL-6, IL-10 and TNF-α ($P<0.05$) and significantly diminished alcohol-induced CYP2E1 activity ($P<0.05$).

Lin CC, Yin MC. 2007. Br J Nutr. 2007. 99(1):37-43.

The intake of *N*-acetyl cysteine (NAC), *S*-ethyl cysteine (SEC) or *S*-propyl cysteine (SPC) treatment significantly suppressed high saturated fat-induced hepatic messenger RNA (mRNA) expression of sterol regulatory element-binding protein (SREBP)-1c and SREBP-2 ($P<0.05$), increased hepatic glutathione content ($P<0.05$), restored the activity and mRNA expression of glutathione peroxidase, and alleviated the high saturated fat-induced oxidative stress ($P<0.05$).

Battal M, Kartal A, et al. 2015. Chirurgia (Bucur). 110(2):117-22.

Supplementation with allyl disulfide, a compound found in AGE, was found to have a positive impact on liver regeneration, proliferation and oxidative damage in models that undergone major hepatectomy.

Chen LY, Chen Q, et al. 2016. Biomed Pharmacother. 79:35-43.

Diallyl trisulfide (DATS) was shown to prevent ethanol-induced injury, as indicated by the reduced activities of aspartate transaminase (AST) and alanine aminotransferase (ALT) in serum of models given a single dose of ethanol *in vivo*, and in culture medium in ethanol-stimulated LO2 cells (*in vitro*) to mimic alcoholic fatty liver (AFL), and inhibition of cell apoptosis. DATS reduced hepatic steatosis and alleviated ethanol-induced oxidative stress.

Al-Brakati A. 2019. Environ Sci Pollut Res Int. [Epub head of print].

AGE administration was found to attenuate the histological deformations and biochemical alteration produced by ethephon. These findings suggests that AGE could be used to reverse the hepatic injury following ethephon exposure through its antioxidant capacity.

Immune Enhancement and Anti-Infection Effects

Immune Enhancement Seen in Clinical Studies

Kohno M, Shirakura T, et al. 1976. Yakuri to Chiryo (Jpn J Pharmacol Ther). 4(3):700-8.

Daily consumption of 4 ml of Leopin-5® (LE-5) significantly increased leukocyte count after 50 days of intake in patients 46-50 years of age participating at an Omiya Camp of the Japanese Land Self-Defense Force.

Kandil OM, Abdullah TH, et al. 1987. Fed Proc. 46(3):441. Abst #723.

Kandil OM, Abdullah TH, et al. 1988. Arch AIDS Res. 1:230-1.

Abdullah TH, Kandil O, et al. 1988. J Natl Med Assoc. 80(4):439-45.

After a 3-week period of garlic intake, AGE (1800 mg) was effective at enhancing natural killer (NK) cell activity. Furthermore, AGE was more effective than a high dosage of raw garlic (0.5 mg/kg body weight for a 70 kg/154 lb. man or equivalent to 35 g/10 cloves of garlic). NK cell activity was enhanced 140% by raw garlic and 160% by AGE.

Abdullah TH, Kirkpatrick DV, et al. 1989. Deutsche Zeitschrift fuer Onkologie. 21:52-3.

AGE enhanced natural killer (NK) cell activity and improved helper/suppressor T cell ratios in AIDS patients. After 6 weeks of intake, NK cell activity was within the normal range for all subjects. Also, patients in this study noted improvements in diarrhea, candidiasis, pansinusitis with recurrent fever and interruption of recurrent cycles of genital herpes.

Ushirotake T, Ushirotake A, et al. 2004. Rinsho Iyaku (Clin Drug Pharmacol). 20(7):785-93.

Following a 2-month examination period between a group taking AGE for more than 1 year and the control group, it was observed that the frequency of catching colds was significantly lower in the AGE group and those in the AGE group who caught the cold recovered from the symptoms quicker compared to the control group.

Ishikawa H, Saeki T, et al. 2006. J Nutr. 136(3 Suppl):816S-20S.

In a randomized, double-blind trial AGE was administered to patients with inoperable colorectal, liver or pancreatic cancer. It was found that both the number of natural killer (NK) cells and NK activity increased significantly in the AGE group.

Nantz MP, Rowe CA, et al. 2012. Clin Nutr. 31(3):337-44.

A randomized, double-blind, placebo-controlled parallel intervention study recruited 120 healthy subjects (60 per group) and given 2.56 g of AGE per day. After 45 days of AGE supplementation, gamma-delta (γδ) T cells ($p=0.039$, $n=56$) and natural killer (NK) ($p=0.043$, $n=56$) were shown to proliferate better compared to placebo. After 90 days, illness diary entries showed the incidence of colds and flu; the group consuming AGE appeared to have reduced the severity as noted by a reduction in the number of days (61% fewer, $p<0.001$) and incidences (58% fewer, $p<0.001$) where the subjects functioned sub-optimally and the number of work/school days missed due to illness (58% fewer, $p=0.035$).

Xu C, Mathews AE, et al. 2018. Clin Nutr ESPEN. 24:148-55.

In a parallel, double-blind, placebo-controlled, randomized study, 51 healthy adults with obesity received a divided daily dose of 3.6 g of AGE or placebo, with food for 6 weeks. Serum interleukin-6 (IL-6) ($p = 0.04$) and tumor necrosis factor-α (TNF-α) ($p = 0.05$) of participants consuming AGE were significantly lower than those consuming placebo. The AGE group had a higher proportion of gamma-delta (γδ) T cells ($p = 0.03$) and a lower proportion of natural killer (NK) T cells ($p = 0.02$) in the total population of lymphocytes. A significant difference in blood low-density lipoprotein (LDL) cholesterol concentration was also observed ($p = 0.05$).

Immune Enhancement Seen in Pre-Clinical Studies

Kyo E, Uda N, et al. 1999. 6th Annual Meeting on Japanese Association for Cancer Prevention. Tokyo, Japan. Jul 16-17. Abst #P-38.

Kyo E. 2000. 6th Annual Meeting of Jpn Mibyou System Assoc. Hiroshima, Japan. Jan 28, p. 43.

AGE inhibited histamine release from mast cells and decreased ear swelling indicating an immunoglobulin E (IgE)-mediated skin reaction. AGE also inhibited the growth of Sarcoma-180 and Lewis Lung Carcinoma (LL/2), and enhanced the number of natural killer (NK) cells and NK activity. Furthermore, AGE inhibited the decreased in spleen weight, cell numbers, anti-sheep red blood cell (SRBC) antibody production, and NK activity induced by psychological stress. All of which indicate that AGE may be useful for maintaining homeostasis of immune function as an immune modulator.

Suzuki J, Yamaguchi T, et al. 2016. Nutrition. 32(7-8):884-9.

Models were orally administered S-1-propenylcysteine (S1PC) (7.5, 15, and 30 mg/kg) for 5 days. S1PC increased the intestinal immunoglobulin (IgA) level and number of IgA-producing cells in Peyer's patches. Furthermore, S1PC induced the expression of X-box binding protein 1 (Xbp1) mRNA, an inducer of plasma cell differentiation, in Peyer's patches, accompanied by the degradation of paried box

protein 5 and the activation of mitogen activated protein/extracellular signal-regulated kinase signaling pathway.

Ramadan G, El-Beih NM, et al. 2017. Environ Toxicol. 32(3):789-98.

Malathion and carbaryl are the most widely used organophosphate and carbamate insecticides, respectively, especially in developing countries. In toxicity induced by malathion and/or carbaryl in models, AGE containing 0.1% *S*-allylcysteine (SAC) (200 mg/kg body weight) for four consecutive weeks. AGE completely modulated most adverse effects induced by malathion and/or carbaryl in models including the normcytic normochromic anemia, immunosuppression, and the delay in the skin-burning healing process through normalizing the count of blood cells (erythrocytes, leucocytes and platelets), hemoglobin content, hematocrit value, blood glucose-6-phosphodehydrogenase activity, weights and cellularity of lymphoid organs, serum γ-globulin concentration, and the delayed type of hypersensitivity response to the control values, and accelerating the inflammatory and proliferative phases of burn-healing. AGE also completely modulated the decrease in serum reduced glutathione (GSH) concentration and the increase in clotting time in malathion alone and carbaryl alone treated models. Moreover, AGE induced a significant increase ($P < 0.001$) in serum GSH concentration (above the normal value) and accelerating burn-healing process in healthy models.

Immune Enhancement Seen in Cell Culture Studies

Hirao Y, Sumioka I, et al. 1987. Phytother Res. 1:161-4.

The F-4 protein fraction and F-3 fructan fraction in AGE stimulated the activities of macrophages and spleen cells in this *in vitro* study.

Lau BHS. 1989. Int Clin Nutr Rev. 9:27-31.

Leukocytes or white blood cells were taken from the peritoneal cavity, spleen and lymph nodes of models following the intake of Kyolic AGE. Using chemiluminescence, it was shown that the phagocytic activity of these cells was significantly enhanced.

Tadi P, Teel RW, et al. 1990. Int Clin Nutr Rev. 10:423-9.

Using chemoluminescence, it was confirmed that the phagocytic activity of AGE and attributed the control of *Candida albicans* in a living model with this effect.

Lau BHS, Yamasaki T, et al. 1991. Mol Biother. 3(2):103-7.

F-4 protein fraction from AGE was found to stimulate the proliferation of T-lymphocytes.

Morioka N, Sze LL, et al. 1993. Cancer Immunol Immunother. 37(5):316-22.

A protein fraction (F-4) isolated from AGE was found to enhance the ability of human peripheral blood lymphocytes to destroy tumor cells. Moreover, F-4 significantly stimulated the lymphokine (interleukin-2 or IL-2)-activated killer activity. F-4 also enhanced the proliferation of lymphocytes induced by the immune-stimulating agents IL-2 and concanavalin-A, suggesting a possible reduction of the dosage of IL-2 in cancer immunotherapy.

Kyo E, Uda N, et al. 1998. Phytomedicine. 5(4):259-67.

Kyo E, Uda N. 1999. 58th Annual Meeting of Japanese Cancer Association. Hiroshima, Japan. Sep 29-Oct 1. Abst #2170.

AGE stimulated the proliferation of model spleen cells and the release of cytokines. AGE also strongly enhanced phagocytosis of peritoneal macrophages and increased natural killer (NK) cell activity both *in vitro* and *in vivo*. After 24 hours, AGE doubled the ability of NK cells to destroy lymphoma cells YAC-1.

Suzuki J, Yamaguchi T, et al. 2016. Nutrition. 32(7-8):884-9.

Model splenic lymphocytes were treated with *S*-1-propenylcysteine (S1PC) (0.1 and 0.3 mM) for 3 days were found to enhance intestinal immunoglobulin A (IgA) production in culture.

Superior Immunostimulatory Properties

Lau BHS, Yamasaki T, et al. 1991. Mol Biother. 3(2):103-7.

AGE and its protein fraction were shown to have significant dose-related augmentation of oxidative burst of J774 macrophage cell line. The protein fraction also enhanced T-lymphocyte blastogenesis. This suggests that garlic compounds may serve as biological response modifiers by augmenting macrophage and T-lymphocyte functions.

Kyo E, Uda N, et al. 1999. In: Immunomodulatory Agents from Plants. Wagner H (ed). Birkhauser Verlag Basel (Switzerland), pp. 273-88.

AGE was shown to enhance proliferation of spleen cells and augment the immune-stimulating activity of various well-known immunostimulatory agents (concanavalin-A [ConA], phytohemagglutin, lipopolysaccharide and interleukin-2 [IL-2]). AGE also increased cytokine production (IL-2, tumor necrosis factor-α [TNF- α] and interferon-γ [IFN-γ]) from spleen cells. It enhanced the activity of natural killer (NK) against lymphoma cells (YAC-1) and also prevented immunosuppression induced by removal of the thymus and showed various anti-tumor activities via immunomodulation. Restoration of stress-induced immune suppression and anti-allergy effects were also noted. Suggested actives such as a protein

fraction and low-weight sugar fractions were mentioned.

Kasuga S, Uda N, et al. 2001. J Nutr. 131(3 Supp):1080S-4S.

The pharmacological activities of four garlic preparations were investigated: raw garlic juice (RGJ), heated garlic juice (HGJ), dehydrated garlic powder (DGP) and AGE. Although all four garlic preparations enhanced natural killer (NK) cells and NK cell activities of the spleen cells of tumor-bearing models, only AGE and HGJ inhibited the growth of inoculated tumor cells.

Indirect Anti-Tumor Effects via Immune Enhancement

Hirao Y, Sumioka I, et al. 1987. Phytother Res. 1:161-4.

The F-4 protein fraction in AGE strongly stimulated peritoneal macrophage activity and exhibited cytostatic activity *in vitro*. F-4 also stimulated the proliferating activity of spleen vells. *In vivo*, F-4 induced the stimulation of carbon clearance activity. F-4 was concluded to be effective for the suppression of tumor cell outgrowth through the stimulation of immunoresponder cells.

Morioka N, Sze LL, et al. 1993. Cancer Immunol Immunother. 37(5):316-22.

A protein fraction (F4) isolated from AGE enhanced the cytotoxicity of human peripheral blood lymphocytes against tumor cells. Moreover, F4 significantly stimulated the lymphokine (interleukin-2 or IL-2)-activated killer activity. F4 also enhanced the proliferation of lymphocytes induced by IL-2 and concanavalin-A (ConA), suggesting a possible reduction of the dosage of IL-2 in cancer immunotherapy.

Riggs DR, Lamm DL, et al. 1995. J Urol. 153(4). Abst #1029.

AGE was shown to be a highly impressive nontoxic oral treatment modality in a bladder cancer model. The effect was similar to that of *Bacillus Calmette-Guerin* (BCG).

Kyo E, Uda N, et al. 1997. UICC Symposium Familial Cancer and Prevention. Kobe, Japan. May 14-16.

Kyo E, Uda N, et al. 1998. Phytomedicine. 5(4):259-67.

Kyo E, Uda N. 1999. 58th Annual Meeting of Japanese Cancer Association. Hiroshima, Japan. Sep 29-Oct 1. Abst #2170.

Kyo E, Uda N, et al. 2001. J Nutr. 131(3 Suppl):1075S-9S.

AGE could be a significant mmune-potentiator and could exhibit anti-tumor activities through immune modulation. After 3 weeks of oral intake, AGE inhibited the growth of Sarcoma-180 by 50% and Lewis Lung cancer cell line (LL/2) by 20%. Killer cell activity of spleen cells against Sarcoma-180 was also significantly enhanced by AGE ($p<0.01$) but not Polysaccharide-K (PSK or Krestin), an anti-cancer drug approved in Japan, and natural killer (NK) cell activity against the cancer cell line YAC-1 was enhanced by both AGE ($p<0.01$) and PSK ($p<0.05$). AGE had no direct effect on LL/2 and Sarcoma-180 *in vitro* confirming that its effects were via immune enhancement and not cytotoxicity.

Riggs DR, Dehaven JL, et al. 1997. Cancer. 79(10):1987-94.

It was found that AGE may help to reduce the development of tumors in the bladders implanted with human bladder cancer cells (MBT2). AGE was also found to have some ability to directly kill MBT2 cells in test tubes. AGE given orally was effective without toxicity or side effects. It reduced tumor incidence by as much as 40% and tumor growth by as much as 60% ($P=0.0002$) and enhanced survival by 50%. Its effects were similar to that of *Bacillus Calmette-Guerin* (BCG), a potent immunomodulatory treatment widely used in human cancer therapy. The authors suggested that AGE might be an excellent adjuvant to traditional treatment.

Inhibited of UV-Induced Immunosuppression

Reeve VE, Bosnic M, et al. 1993. Phytochem Phytobiol. 58(6):813-7.

Reeve VE, Bosnic M, et al. 1997. Ch. 17. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 163-75.

Reeve et al. found that ultraviolet (UV) light exposure caused a 58% suppression of systemic contact hypersensitivity (SCH), an immune response. When the models were fed 4% AGE, the immune response was suppressed by only 19%. Thus, AGE helped to ameliorate the immunosuppressive effects of UV exposure. When UV exposure was replaced by topical application of cis urocanic acid-containing lotions, which also suppress SCH, AGE completely protected the models from this form of immunosuppression.

Anti-Viral Effects Against Influenza

Nagai K. 1973. Kansenshogaku-Zasshi (Jpn J Infect Dis). 47(9):321-5.

In models that were per nasally inoculated with influenza virus AO/PR 8, AGE was found to enhance the effectiveness of an influenza vaccine and when used alone, was found to be as effective as the vaccine.

Nagai K. 1973. Kansenshogaku-Zasshi (Jpn J Infect Dis). 47(4):111-5.

By giving either AGE alone or Kyoleopin® to models for 15 days prior to per nasal inoculation with influenza virus significantly improved their outcome.

Anti-Microbial Activity

Kodera Y, Matsuura H, et al. 1989. Chem Pharm Bull. 37(6):1656-8.

Kodera et al. isolated a phenolic stress compound from garlic and termed it allixin. Allixin was found to possess a weak antimicrobial activity.

Kodera Y, Ayabe M, et al. 2002. Chem Pharm Bull. 50(3):405-7.

Allixin, a *de novo* synthesized substance categorized as a phytoalexin, may pose a prohibitory, inhibitory or post-inhibitory antimicrobial function in garlic. The basis of this conclusion comes from the observation of high accumulation of allixin in nectrotic tissue areas after long-term storage.

Anti-Allergy

Kyo E. 1998. 28th Annual Meeting of the Japanese Society for Immunology. Dec 2-4. Abst #3-A3-082.

Three systems, *in vitro* histamine release system, *in vivo* immunoglobulin E (IgE)-mediated skin reaction system and *in vivo* late phase reaction system, were used to determine the effect of AGE has on mast cells and activated T-lymphocyte function. AGE was found to modify allergic cascade reactions (i.e., inflammation).

Reduced Inflammation Associated with Allergy

Kyo E, Uda N, et al. 1997. Phytomedicine. 4(4):335-40.

It was demonstrated that an allergic-type reaction by adding compounds (anti-trinitrophenyl [TNP] monoclonal antibody and trinitrophenyl-bovine serum albumin [TNP-BSA]) hapten carrier complex) to the immune cells basophile cell line RBL-2H3 causes them to release histamine. When AGE was additionally added at increasing dosages (1.25, 2.5 and 5.0 v/v%), it significantly inhibited histamine release by 50%, 80% and 90%, respectively. The anti-allergy drug oxatomide (10 mcg/ml) inhibited histamine release by 80%.

In another experiment by Kyo et al., AGE given orally (10 ml/kg) to models showed anti-inflammatory effects by decreasing ear swelling induced by the topical administration of a known skin irritant picryl chloride and intravenous administration of anti-TNP immunoglobulin E (IgE) antibody that induces skin reactions, a type I allergic reaction. AGE reduced swelling by 25-45%, thus decreasing the allergic reaction triggered by mast cells.

When AGE and oxatomide were administered directly into the stomach 1 hour following a picryl chloride application to the ears of models, oxatomide reduced swelling by 47% and AGE reduced it by 19%. However, 4 and 24 hours following application, AGE out-performed oxatomide.

In the final experiment, AGE was found to inhibit a type IV allergic reaction. Picryl chloride was first applied to abdominal skin and then subsequently to the ears of models 7 days later. Either dexamethasone, a known immunosuppressor, or AGE was then administered orally 0, 4 and 16 hours (3 times or 1 time at each respective hour). Ear thickness was measured 24 hours after the secondary challenge. Repeated oral administration of dexamethasone suppressed ear swelling by 65% and AGE by 55%.

As shown in these experiments, AGE may reduce allergic-type reactions.

Kyo E, Uda N, et al. 2001. J Nutr. 131(3 Suppl):1075S-9S.

In an immunoglobulin E (IgE)-mediated allergic model, AGE was shown to significantly decrease the antigen-specific ear swelling, which was induced by an application of picryl chloride ointment to the ear and an intravenous administration of anti-trinitrophenyl antibody IgE ascites.

Zare A, Farzaneh P, et al. 2008. Iran J Allergy Asthma Immunol. 7(3):133-41.

AGE caused a significant decrease in the hallmark criteria of allergic airway inflammation levels which included eosinophil percentage in lavage, peribronchial lung eosinophils, immunoglobulin G1 (IgG1) level in lavage and serum, mucus producing goblet cells grade and peribronchial and perivascular inflammation. These results suggest that AGE has the potential of attenuation of inflammatory features of allergic airway inflammation in models.

Shin NR, Kwon HJ, et al. 2019. Int Immunopharmacol. 68:124-30.

In an ovalalbumin (OVA)-induced asthma model, *S*-allylcysteine (SAC) was shown to effectively suppress allergic airway inflammation and mucus production, which shows potential for use in treating allergic asthma.

Improved Immune Function and Depressed State of Post-Menopause

Mizuno I, Yasuda K, et al. 2005. J Jpn Mibyou System Assoc. 11:117-

Leopin Royal® (LER) was evaluated using post-menopausal models. Removal of ovaries caused a state of

21.

Mizuno I, Yasuda K, et al. 2005. 11th Annual Meeting of Jpn Mibyou System Assoc. Saitama, Japan. Jan 8-9. Abst #27.

depression and immune function disorder, however, when supplemented with LER, models showed improvement in the depressed state and interferon-γ (IFN-γ) production, an index of immune function. This data indicates that LER improves the disorder of nerve-internal secretion immune network induced by the ovary dysfunction and suggests it may be useful for women during menopause.

Improved Age-Related Deterioration of Immune Responses

Zhang Y, Moriguchi T, et al. 1997. Ch. 13. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 117-29.

Chronic oral administration of AGE significantly improved the suppression of antibody production caused by removal of the thymus gland from two different models with genetically prone to accelerated aging (senescence-accelerated prone mouse or SAMP8). AGE also restored levels of hypothalamic norepinephrine 3,4-dihydroxyphenylacetic acid and homovanilic acid, and the hypothalamic choline acetyltransferase activity, which were significantly increased by removal of the thymus. Chronic ingestion of AGE significantly enhanced white blood cell production activated by the immune-stimulating agents concanavalin A or lipopolysaccharides in both SAMP8 and senescence accelerated mouse resistant 1 (SAMR1), a normal substrain of SAM not genetically prone to accelerated aging.

Immune Review

Percival SS. 2016. J Nutr. 146(2):433S-436S.

Supplementation with AGE may enhance immune cell function and may be partly responsible for the reduced severity of colds and flu reported as shown in one randomized, double-blind, placebo-controlled parallel-intervention study in which healthy adults consumed 2.56 g of AGE daily for 90 days during thre cold and flu season. After 45 days of AGE consumption, two innate lymphocytes gamma-delta (γδ) T and natural killer (NK) cells proliferated better and were more activated than cells from the placebo group. After 90 days, AGE group showed reduced cold and flu severity, with a reduction in the number of symptoms, the number of days participants functioned suboptimally, and the number of work/school days missed.

Rodrigues C, Percival SS. 2019. Nutrients. 11(2):pii:E295.

Glutathione and AGE are sulfur-containing products that play important protective and regulatory roles within the immune system and in oxidative processes. Recent studies have shown that sulfur-containing compounds from garlic have beneficial effects in attenuating outcomes associated with cardiovascular disease and inflammation by a mechanism that may be related to the H_2S signaling pathway. The main functions of glutathione (GSH), garlic derivatives and H_2S and their role in t he immune response and impact on health and disease are discussed in this review.

Suzuki JI, Miki S, et al. 2020. Exp Ther Med. 19(2):1570-3.

This review summarizes the mechanisms through which the activation of autophagy by *S*-1-propenylcysteine (S1PC), a characteristic sulfur compound in AGE, modulates the immune response.

Overview of Immune-Enhancing Effects of AGE and Its F-4 Protein Fraction			
Clinical	IMMUNE-ENHANCING	AGE	F-4 FRACTION
	Severity of cold/flu		
	- The number of days a person suffers from cold/flu[1]	↓ 61%	
	- The number of symptoms of cold/flu[1]	↓ 21%	
	- The number of work days missed due to their illness[1]	↓ 58%	
	Gamma delta T (γδT) cells[1,5]	↑ 13-700%	
	Natural killer (NK) cells[1-4]	↑ 32-567%	
	Natural killer T (NKT) cells[5]	↓ 25%	
	Interleukin-6 (IL-6)[5]	↓ 26%	
	Tumor necrosis factor-alpha (TNF-α)[5]	↓ 0.7%	
Non-clinical	Interferon-gamma (IFN-γ)[6,7]	↑ 80%	
	Interleukin-2 (IL-2)[6]	↑ 200%	
	Lymphocytes[7]		↑ 30-160%
	Macrophages[8]	↑ 110-290%	↑ 250%
	Phagocytes[9]	↑ 40-900%	
	T-cells[8]		↑ 60%
	Tumor necrosis factor-alpha (TNF-α)[6,7]	↑ 330-850%	

1. Nantz MP, Rowe CA, et al. 2012. Clin Nutr. 31(3):337-44.
2. Abdullah TH, Kirkpatrick DV, et al. 1989. Deutsche Zeitschrift fuer Onkologie. 21:52-3.
3. Ishikawa H, Saeki T, et al. 2006. J Nutr. 136(3 Suppl):816S-20S.
4. Kandil OM, Abdullah TH, et al. 1987. Fed Proc. 46(3):441. Abst #723.
5. Xu C, Mathews AE, et al. 2018. Clin Nutr ESPEN. 24:148-55.
6. Kyo E, Uda N, et al. 1999. In: Immunomodulatory Agents from Plants. Wagner H (ed). Birkhauser Verlag Basel (Switzerland), pp. 273-88.
7. Morioka N, Sze LL, et al. 1993. Cancer Immunol Immunother. 37(5):316-22.
8. Lau BHS, Yamasaki T, et al. 1991. Mol Biother. 3(2):103-7.
9. Lau BHS. 1989. Int Clin Nutr Rev. 9:27-31.

Antioxidative and Radio-Protective Effects

Borek C. 2001. J Nutr. 131(3 Suppl):1010S-5S.

Oxidation modification of DNA, proteins and lipids by reactive oxygen plays a role in aging including cardiovascular, inflammatory disease and cancer. Garlic and garlic extracts contains antioxidant nutrients and phytochemicals that prevent oxidant damage via different and complementary mechanisms; scavenging reactive oxygen and increasing inherent defenses. Aging the garlic extract increases their antioxidant potential, in part by amplifying stable bioavailable organosulfur compounds. AGE contains antioxidants that protect DNA against free radical mediated damage and mutations; defend against radiation and sunlight damage and inhibit multi-step carcinogenesis. AGE also inhibits lipid peroxidation, maintains blood vessel integrity, increases blood flow and reduces the risk of atherosclerosis and cardiovascular diseases that are accelerated by low-density lipoprotein (LDL) peroxidation. AGE supplementation amplifies and strengthens the antioxidant network in cells and has wide reaching health potentials in defending the body against age-related ailments, including inflammatory conditions and oxidant-mediated damage to the brain, helping preserve brain function and a healthy life.

Borek C. 2005. J Nutr. 136(3 Suppl):810S-2S.

AGE is helpful in reducing risk factors of cardiovascular disease, including high cholesterol which is associated with dementia and Alzheimer's disease. AGE is able to scavenge oxidants, inhibit cholesterol synthesis and help prevent cognitive decline by protecting neurons from β-amyloid (Abeta) neurotoxicity and apoptosis.

Morihara N, Ide N, et al. 2005. Redox Rep. 10(3):159-65.

When AGE is combined with a suspension of erythrocytes, it decreased peroxynitrite-induced hemolysis in a concentration-dependent manner. *S*-allylcysteine (SAC), a compound found in AGE, was also found to decrease hemolysis. Since peroxynitrite is a strong oxidant, it has been shown to cause vascular or tissue damage. Therefore, AGE and its constituents may be helpful for preventing cardiovascular diseases and may help prevent the damage of membranes in erythrocytes.

Chung LY. 2006. J Med Food. 9(2):205-13.

Through chemical synthesis or purification of the four main chemical classes of garlic compounds: allyl cysteine, alliin, allicin and allyl disulfide; each one exhibited different patterns of antioxidant activities as protective compounds against free radical damage in the body. Alliin and allyl cysteine were shown as hydroxyl radical scavenger while allicin had no effect.

Chen CM, Yin MC, et al. 2007. Nutrition. 23(7-8):589-97.

Pre-intake of *N*-acetyl cysteine (NAC), *S*-ethyl cysteine (SEC), *S*-methyl cysteine (SMC) and *S*-propyl cysteine (SPC) significantly attenuated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced glutathione loss, retained the activity of glutathione peroxidase (GPX) and superoxide dismutase, diminished oxidative stress and suppressed MPTP-induced elevation of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) ($P<0.05$) in models. The four cysteine-containing compounds also significantly elevated GPX messenger RNA (mRNA) expression and diminished TNF-α mRNA expression ($P<0.05$), improved MPTP-induced dopamine depletion and increased dopamine/3,4-dihydroxyphenylacetic acid content ($P<0.05$), in which these results suggest that NAC, SEC, SMC and SPC could provide antioxidative and anti-inflammatory protection for the striatum against the development of Parkinson's disease.

Ogawa T, Kodera Y, et al. 2016. Sci Rep. 6:21611.

The garlic derived thioallyl compounds *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC) were shown to increase the lifespan and stress resistance in Caenorhabditis elegans and reduce accumulation of reactive oxygen species (ROS). They selectively induce SKN-1 (Nrf1/2/3 orthologue) targets involved in oxidative stress defense although, interestingly, their treatments do not facilitate SKN-1 nuclear accumulation, but slightly increased intracellular SKN-1 levels. The data also indicate that thioallyl structure and the number of sulfur atoms are important for SKN-1 target induction.

Orozco-Ibarra M, Munoz-Sanchez J, et al. 2016. Biol Res. 49:7.

The effect of AGE and *S*-allylcysteine (SAC) were assessed on cobalt chloride (CoCl2)-chemical hypoxia model in PC12 cells. Treatment with AGE and SAC decreased reactive oxygen species (ROS) and protected against CoCl2-induced apoptotic cell death which depended on the CoCl2 concentration and incubation time. SAC or AGE decreased the number of cells in the early and late stages of apoptosis, in which was associated with attenuation in hypoxia inducible factor (HIF-1α) stabilization, an activity not previously reported for AGE or SAC.

Dvorakova M, Heroutova I, et al. 2016. PeerJ. 4:e2280.

S-allylcysteine (SAC) (0.1, 0.5 and 1.0 mM) was shown to reduce levels of reactive oxygen species (ROS) in maturing oocytes significantly after 24 h (reduced by 90.33, 82.87 and 91.62%, respectively), and 48 h (86.35, 94.42 and 99.05%, respectively) cultivation, without leading to a disturbance of the standard

course of meiotic maturation. Oocytes matured in the presence of SAC furthermore maintained reduced levels of ROS even 22 h after parthenogenic activation (reduced by 66.33, 61.64 and 57.80%, respectively). A growth of early embryo cleavage rate (increased by 33.34, 35.00 and 35.00%, respectively) was also demonstrated in these oocytes.

Protection from Radiation and Chemotherapy

Toriyama M. 1983. Nihon Jibirinsho Zasshi (Jpn J Jibirinsho). 76(2):231-40.

Kyoleopin® (KLE) reduced the side effects associated with head and neck tumors. Twelve of 30 patients, either on radiotherapy or chemotherapy, suffering from head and neck tumors found that consumption of KLE for 8 weeks assisted in alleviating anorexia and fatigue while enhancing their will to fight their disease.

Lau BHS. 1989. Int Clin Nutr Rev. 9:27-31.

Kyolic AGE protected cultured lymphocytes from irradiation damage. Fresh garlic, on the other hand, had no protective effect. Rather, it worsened irradiation damage moreso than irradiated controls receiving no treatment.

Horie T, Matsumoto H, et al. 1999. Planta Med. 65(6):545-8.

Horie T, Awazu S, et al. 2001. J Nutr. 131(3 Suppl):1071S-4S.

Anti-tumor drugs like 5-fluorouracil (5-FU) and methotrexate (MTX) induce intestinal damage, a serious side effect of cancer chemotherapy. 5-FU or MTX was orally administered for 4 or 5 days to models fed with the standard diet with and without AGE. The small intestine absorption of a poorly absorbable compound, fluoracin isothiocyanate-labeled dextran (FD-4) was examined to evaluate damage to the intestine using the *in vitro* everted intestine technique and the *in situ* intestinal loop technique. The FD-4 absorption through the small intestine increased in the anti-tumor drug-treated models fed with the diet without garlic suggesting intestinal damage. However, absorption decreased with the diet containing AGE almost to the same level as the models receiving no MTX. These results suggest that AGE protects the small intestine from anti-tumor drug-induced damage.

Protection from Gentamicin-Nephrotoxicity

Maldonado P, Barrera D, et al. 2003. Life Sci. 73(20):2543-56.

AGE has been shown to prevent nephrotoxicity by: decrease in blood urea nitrogen and plasma creatinine, increase in plasma glutathione peroxidase (GPx) activity and urinary decrease in *N*-acetyl-β-D-glucosaminidase (NAG) activity and total protein. In addition, AGE prevented gentamicin (GM)-induced increase in the renal levels of oxidative stress markers: nitrotyrosine and protein carbonyl groups and the decrease in manganese superoxide dismutase (Mn-SOD), GPx and glutathione reductase (GR) activities.

Maldonado PD, Chanez-Cardenas ME, et al. 2005. Phytother Res. 19(3):252-4.

In this study, AGE, garlic powder extract (GPE), *S*-allylcysteine (SAC), diallyl sulfide (DAS) or diallyl disulfide (DADS) were assessed for their ability to interfere with the *in vitro* antibiotic activity of gentamicin (GM) in *Escherichia coli* (*E. coli*) cultures. Although the above mentioned extracts and compounds of garlic were unable to decrease the antibiotic capacity of GM, but SAC, DAS and DADS alone inhibited the growth of *E. coli* and enhanced the antibiotic effect of GM. It is suggested that AGE, GPE, SAC, DAS and DADS may be administered along with GM to alleviate GM-induced nephrotoxicity without interfering with its antibiotic activity.

Valasco-Velazquez MA, Maldonado PD, et al. 2006. Phytother Res. 20(1):76-8.

AGE at 1% produced maximum protective effects over gentamicin (GM) toxicity, while only partial protection was observed in GM-genotoxicity at the same concentration. GM-induced toxicity could not be prevented with *S*-allylcysteine (SAC) at different concentrations (0.5-8 mM); however, SAC demonstrated protective effects on cells with GM-induced genotoxicity. Only AGE was able to stimulate cell proliferation in both treated and untreated samples.

Protection from Gentamicin-Ototoxicity

Uzun L, Kokten N, et al. 2016. Clin Exp Otorhinolaryngol. 9(4):309-13.

Gentamicin is a potent aminoglycoside antibiotic in which ototoxicity and nephrotoxicity are the main side effects. Models injected with gentamicin and treated with either *S*-allylmercaptocysteine (SAMC) (Genta-w SAMC), diallyl disulfide (DD) (Genta-w DD), *S*-allylcysteine (SAC) (Genta-w SAC), gentamicin without any active compounds (AC) (Genta-w/o AC), or control. Using the brainstem evoked response audiometry (BERA) test, the mean amplitude of auditory thresholds (sensation level [SL]) for the groups were 22±8, 25±5, 30±9, 54±11, and 10±7 dB SL, respectively. The differences between every active compound group (Genta-w SAMC, Genta-w DD, and Genta-w SAC) and Genta-w/o AC were statistically significant ($P<0.016$).

Attenuates Ivermectin-induced Cytogenotoxicity in Bone Marrow Cells

Khalil AM, Abu Samrah HM. 2018. Res Vet Sci. 120:94-100.

Ivermectin (IVM) is widely used in human and veterinary medicine for the control of parasitic infections, were shown to induce significant levels of cytogenetic toxicity at doses higher than the recommended dose. Models were given the minimum detectable toxic (MDT) dose of IVM at 0.4 mg/kg body weight (b w) and at varying doses of AGE; 300, 600 and 1200 mg/kg b w. AGE at 1200 mg/kg demonstrated a powerful capacity to reduce IVM cytogenetic effects by significantly elevating mitotic index (MI) in bone marrow cells, indicating that AGE was able to mitigate the inhibitory effect of IVM on cell division.

Reduces Antioxidative Damage in Smokers in Clinical Studies

Dillon SA, Lowe GM, et al. 2002. J Nutr. 132(2):168-71.

Dillon SA, Lowe GM, et al. 2002. International Research Conference on Food Nutrition and Cancer. Washington, D.C. Jul 11-12. J Nutr. 132(11 Suppl):3549S.

Dietary supplementation with Kyolic AGE at 5 ml per day for 14 days reduced concentrations of a marker of lipid peroxidation 8-iso-prostaglandin-2Fα (8-iso-PGF2α) by 29% and 37% in plasma and urine, respectively, in nonsmokers and by 35% and 48%, respectively, in smokers. Two weeks after cessation of dietary supplementation, plasma and urine concentrations returned to normal. Thus, dietary supplementation with AGE may be useful in reducing oxidative stress in humans.

In vitro Protection from Lipid Peroxidation

Horie T, Murayama T, et al. 1989. Planta Med. 55(6):506-8.

When free radicals attack cell membranes, which are high in lipids or fat, they form lipid peroxides. Lipid peroxidation seems to be closely related to toxicity, disease and aging. Thiobarbituric acid reactive substances (TBA-RS) are used as markers of lipid peroxidation and when AGE was added to liver cells, there was a reduction in TBA-RS suggesting an antioxidant effect of AGE. Specifically, at 40 mg/ml AGE completely inhibited the oxidant ascorbic acid/$FeSO_4$-induced lipid peroxidation of liver tissue.

Horie T, Awazu S, et al. 1989. Planta Med. 58(5):468-9.

Additional research found that polysulfide fraction of AGE also significantly prevented lipid peroxidation of liver microsomes.

Horie T. 1990. First World Congress on the Health Signficance of Garlic and Garlic Constituents. Washington, D.C. Aug 28-30, p. 23.

AGE added to liver tissue exposed to peroxidation prevented oxidation as indicated by prevention of the formation of thiobarbituric acid reactive substances (TBA-RS). AGE also prevented the decrease in membrane fluidity caused by peroxidation.

Yang C, Yasaei P, et al. 1993. J Food Drug Anal. 1(4):357-64.

Kyolic AGE at 50 mg afforded protection from lipid oxidation. When Kyolic AGE was added to a methyl linoleate, a standard lipid/fat, held at 60°C (140°F), it reduced the heat induced oxidation of this fat as indicated by a decrease in methyl linoleate hydroperoxide, an intermediate in the lipid oxidation process. AGE also scavenged peroxide radicals.

Ide N, Ryu K, et al. 1996. 2nd International Congress on Phytomedicine. Munich, Germany. Sep 11-14. Abst #SL-109.

Amagase H, Matsuura H, et al. 2000. Ch. 6. In: Phytochemicals and Phytopharmaceuticals. AOCS Press. Champaign, IL, pp. 62-78.

AGE and two of its constituents *S*-allyl cysteine (SAC) and *S*-allyl mercaptocysteine (SAMC) decreased emissions of low level chemiluminescence (LLC) initiated by the oxidant t-butyl hydroperoxide in liver tissue. On the other hand, water extracts of raw and heat-treated garlic enhanced such emissions. AGE reduced emissions by 30% whereas a water extract of raw garlic enhanced emissions by 110% (Amagase et al.). AGE, SAC and SAMC scavenged hydrogen peroxide *in vivo* and AGE was more effective than raw garlic juice.

Ohnishi ST, Kojinra R. 1997. Ch. 12. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 105-15.

Kyolic AGE and *S*-allyl cysteine (SAC) could inhibit the oxidation or rancidity of vegetable oil (a linoleic acid micelle suspension) caused by the radical generator or oxidant 2,2'-azo-bis(2-amidinopropane) HCl (AAPH). When AGE or SAC were added to this oil, they prevented the oxidizing effects of AAPH. In this biologically relevant reaction, AGE was found to be more effective than pure SAC alone at inhibiting lipid peroxidation.

Yamasaki T, Lau BHS. 1997. Nippon Yakurigaku Zasshi (Folia Pharmacol Jpn). 110:138-41.

Yamasaki T. 1997. 25th Pharmacological Activity Symposium. Tokyo, Japan. Oct 30-31.

The effect of AGE on hydrogen peroxide (H_2O_2)-induced oxidant injury was studied and cell viability, lactate dehydrogenase (LDH) release and thiobarbituric acid reactive substances (TBA-RS or lipid peroxidation) were assessed. Results indicate that in a dose-dependent manner, *S*-allylcysteine (SAC) and AGE inhibited LDH release and TBA-RS production with 50 µM of H_2O_2, demonstrating that SAC and AGE have antioxidant properties that can protect vascular endothelial cells from oxidant injury.

Horie T. 2000. 6th Annual Meeting of Jpn Mibyou System Assoc. Hiroshima, Japan. Jan 28, p. 42.

Incubation with isolated microsome, iron and ascorbic acid caused lipid peroxidation in *in vitro* systems. Co-incubation with the aforementioned compounds and garlic compounds, such as diallyl polysulfides, has shown strong antioxidant effects and the ability to inhibit lipid peroxidation.

Ide N, Ryu K, et al. 2000. 54th Annual Meeting of Japanese Society of Nutrition and Food Science. Matsuyama, Japan. May 12-14. Abst #2H-07a.

Incubation of human low-density lipoproteins (LDL) with copper ion for 24 hours increased lipid peroxidation, however, AGE and its compounds were able to inhibit lipid peroxidation. Incubation of endothelial cells with oxidized LDL (Ox-LDL) for 24 hours caused an increase in lactate dehydrogenase (LDH) release, an index of cell membrane damage, thiobarbituric acid reactive substances (TBA-RS), an indication of lipid peroxidation, and a loss in cell viability. However, the opposite effect was seen when endothelial cells were pre-treated with AGE or its compounds, suggesting that it may be useful for the prevention of cardiovascular disease due to it antioxidant effects.

Sumioka I, Ide N, et al. 2000. 54th Annual Meeting of Japanese Society of Nutrition and Food Science. Matsuyama, Japan. May 12-14. Abst #2H-06a.

Antioxidant effects of three garlic preparations including raw garlic extract, boiled garlic extract and AGE, were determined using several *in vitro* systems. Incubation with microsome and t-butyl hydroperoxide enhanced ultra weak chemiluminescence indicating lipid peroxidation. Out of the three garlic preparations, only AGE inhibited lipid peroxidation in this system. AGE and its constituents have shown scavenging effects on hydrogen peroxide. Fructosyl-arginine, a component of AGE, proved 1/10 as strong as the common antioxidant ascorbic acid.

Dillon SA, Burmi RS, et al. 2003. Life Sci. 72(14):1583-94.

Human low-density lipoproteins (LDL) were isolated and challenged with a range of oxidants either in the presence of AGE or its diethyl ether extract. Results of this study indicate AGE's ability to inhibit the *in vitro* oxidation of LDL by scavenging superoxide and inhibiting the formation of lipid peroxides. AGE also reduced LDL oxidation by chelating copper ions. Thus, AGE may have a role in preventing the development and progression of atherosclerotic disease.

Padmanabhan M, Prince PS. 2006. Toxicology. 224(1-2):128-37.

Cell membrane damage in myocardial infarction-induced models increased enzymatic leakage, lipid peroxidation and free radical formation. Oral pretreatment with *S*-allylcysteine (SAC) (100 mg/kg and 150 mg/kg) improved superoxide dismutase, catalase, glutathione reductase and ascorbic acid enzymatic activities. End measures of lipid peroxidation or thiobarbituric acid reactive substances (TBA-RS), were decreased with SAC oral pretreatment. Therefore, improvements in lipid peroxide markers (decrease) and antioxidant status (increase) are due to the antioxidant effect of SAC.

Perez-De La Cruz V, Gonzalez-Cortes C, et al. 2006. Brain Res Bull. 68(5):379-83.

The antioxidant properties of *S*-allyl cysteine (SAC) were examined on lipid peroxidation and mitochondrial dysfunction induced by 3-nitropropionic acid (3-NPA), a neurotoxin. Concentrations of 3-NPA at 0.75-2.5 mM produced enhanced levels of lipid peroxidation while increasing concentrations of SAC (0.1-2 mM) which decreased the peroxidative effects of 3-NPA. SAC at 0.75 mM also prevented 3-NPA (1 mM)-induced mitochondrial dysfunction. It was determined that the protective actions of SAC on 3-NPA-induced lipid peroxidation and mitochondrial dysfunction are due to its antioxidant properties.

In vivo Protection from Lipid Peroxidation

Horie T. 1990. First World Congress on the Health Significance of Garlic and Garlic Constiutents. Washington, D.C. Aug 28-30, p. 23.

AGE was used prior to treatment of carbon tetrachloride (CCl_4), a potent liver toxin, which protected liver tissue from oxidative damage as indicated by a reduction in thiobarbituric acid reactive substances (TBA-RS).

Ide N, Ryu K, et al. 1996. 2nd International Congress on Phytomedicine. Munich, Germany. Sep 11-14. Abst #SL-109.

AGE inhibited *in vivo* lipid peroxidation as indicated by an 80% decrease in pentane production following carbon tetrachloride (CCl_4)-induced lipid peroxidation.

Herrera-Mundo MN, Silva-Adaya D, et al. 2006. Neurosci Res. 56(1):39-44.

The effects of *S*-allyl cysteine (SAC) on early behavioral alterations, striatal changes in superoxide dismutase activity, lipid peroxidation and mitochondrial dysfunction induced by the systemic infusion of 3-nitropropionic acid (3-NPA) to models. SAC given to models 30 minutes before 3-NPA prevented the hyperkinetic pattern by the toxin. 3-NPA alone produced decreased activities of manganese and copper/zinc-dependent superoxide dismutase, increased lipid peroxidation and mitochondrial dysfunction in the striatum. Pre-treatment of 3-NPA-injected models with SAC resulted in a significant prevention of all these markers.

Enhanced Antioxidant Systems in the Body

Geng Z, Lau BHS. 1997. Phytother Res. 11:54-6.

Geng et al. found that AGE could protect cells that line the veins and arteries from damage caused by free radicals by enhancing the activity of the antioxidant enzymes glutathione (GSH) and superoxide dismutase (SOD) in these cells. GSH and SOD are potent intracellular antioxidants while GSH is also a detoxifier. AGE was shown to time- and dose-dependently enhance intracellular GSH level, glutathione

disulfide reductase and SOD.

Wei Z, Lau BHS. 1998. Nutr Res. 18(1):61-70.

The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) play an important role in scavenging oxidants and preventing cell injury. Wei et al. found that AGE enhanced the activity of these three enzymes and thus, suppressed the generation of free radicals, hydrogen peroxide and superoxide anion in pulmonary artery endothelial cells.

O'Brien J, Gillies DG. 1998. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. Nov 15-17, p. 66.

Ryu K, Ide N, et al. 2001. J Nutr. 131(3 Suppl):972S-6S.

Ryu K, Ide N, et al. 2001. Joint ACS-IcoFF Symposiium on Food Factors for Health Promotion. San Diego, CA. Apr 1-5. Abst #AGFD 51.

Two research teams lead by O'Brien et al. and Ryu et al. reported unique Maillard reaction products in AGE. These include *N*-fructosyl glutamate and *N*α-fructosyl arginine (Fru-Arg), which have antioxidant activity. They were generated through a non-enzymatic reaction of amino acids and reducing sugars during the aging of garlic. Fru-Arg completely scavenged hydrogen peroxide, a potent oxidant, at a very low dosage of 50 µM. Since Fru-Arg was detected in high levels in AGE, but not in raw or boiled garlic, its presence may partly explain the antioxidative effects of AGE not shown by other forms of garlic.

Ide N, Lau BHS, et al. 1999. J Nutr Biochem. 10(6):372-6.

Ide N, Ryu K, et al. 2001. 7th International Symposium on the Maillard Reaction. Kumamoto, Japan. Oct 29-Nov 1, p. 18.

*N*α-fructosyl arginine (Fru-Arg) was found to significantly inhibit the oxidizing effects of the copper ion (Cu^{2+}) on low-density lipoprotein (LDL) cholesterol when incubated together as shown by a reduction in thiobarbituric acid reactive substances (TBA-RS) formation. Pretreatment of pulmonary artery endothelial cells with Fru-Arg inhibited cell damage caused by oxidized LDL as indicated by a reduction in lactate dehydrogenase (LDH) release, an indicator of cell damage, and a reduction in TBA-RS formation. Incubation of Fru-Arg with macrophage immune cells also dose-dependently reduced the ability of oxidized LDL to initiate peroxide release from the macrophages. In a cell-free test tube system, Fru-Arg was shown to scavenge the hydrogen peroxide free radical.

Kalayarasan S, Sriram N, et al. 2008. J Appl Toxicol. 28(7):908-19.

In potassium dichromate-induced apoptosis and oxidative stress in hepatocytes of models, administration of AGE and SAC restored liver marker enzymes such as aspartate transaminase, alanine transaminase and lactate dehydrogenase to near normal status. AGE and SAC also reversed the decrease of enzymic antioxidants (superoxide dismutase, catalase, glutathione peroxidase), non-enzymic antioxidants (vitamin C and vitamin E) and the levels of reduced glutathione, while decreasing lipid peroxidation (LPO) and reactive oxygen species in liver tissues. The expression of nuclear factor-E2 related factor 2 (Nrf2), was activated showing a promising role of Nrf2-mediated antioxidant defense of AGE and SAC against chromium toxicity.

Badr GM, Al-Mulhim JA. 2014. Evid Based Complement Alternat Med. doi: 10.1155/2014/759642.

Models were divided into 6 groups: control, indomethacin (IN)-induced gastric inflammation via oral single dose (30 mg/kg to fasted models), two AGE orally administered groups (100 and 200 mg/kg for 30 consecutive days), two AGE orally administered to models pretreated with IN at the same aforementioned doses. The higher AGE dose (200 mg/kg) had more potent effect compared to 100 mg/kg dose as reflected by significant gastric mucosal healing of damage and reduction in the total microbial induced due to IN administration. In addition to the significant effect to normalize the significant increase in malondialdehyde (MDA), myeloperoxidase (MPO), tumor necrosis factor-α (TNF-α) values, and the significant decrease in the total glutathione (tGSH), superoxide dismutase (SOD), and catalase (CAT) values induced by IN.

Hiramatsu K, Tsuneyoshi T, et al. 2016. Nutr Res. 36(2):143-9.

AGE was found to promote the accumulation of nuclear factor erythroid 2-related factor 2 (Nrf2) into the nucleus of human umbilical vein endothelial cells in culture in a time and dose-dependent manner and increased gene expression and polypeptide level of antioxidant enzymes heme oxygenase-1 (HO-1) and glutamate-cysteine ligase modifier subunit (GCLM), via activation of the Nrf2-antioxidant response element (ARE) signaling pathway.

Yamato O, Tsuneyoshi T, et al. 2018. BMC Vet Res. 14(1):373.

Administration of AGE (45 or 90 mg/kg body weight once a day) for 12 weeks in models upregulated the gene expressions of nuclear factor erythroid 2-related factor 2 (Nrf2) and Nrf2-regulated phase II antioxidant enzymes.

Scavenging Effects

Ide N, Matsuura H, et al. 1996. Phytother Res. 10:340-1.

AGE and three of its constituents *S*-allylcysteine (SAC), *S*-allylmercaptocysteine (SAMC) and alliin, demonstrated a scavenging effect on hydrogen peroxide. They also inhibited the chain oxidation induced by a hydrophilic radical initiator. Hydrogen peroxide yields a free radical by reacting with iron or copper

(Fenton reaction). This free radical damages both membranes and DNA and/or induces lipid peroxidation.

Ohnishi S, Kojinra R. 1997. Ch. 12. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 105-15.

AGE and *S*-allyl cysteine (SAC) was shown to inhibit the auto-oxidation of pyrogallol caused by superoxide radicals suggesting that both AGE and SAC could quench superoxide radicals. In addition, AGE and SAC quenched free radicals generated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) in an ethanol solution.

Pietta PG, Simonetti P. 1998. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. Nov 15-17, p. 67.

The total antioxidant activity (TAA – the capacity to scavenge all species of free radicals) of garlic preparations were examined using the Trolox Equivalent Antioxidant Capacity (TEAC) method. AGE showed a significant in vitro antioxidant capacity, which indicate that scavenging of free radicals plays an important role.

Ide N, Ichikawa M, et al. 2001. Joint ACS-IcoFF Symposium on Food Factors for Health Promotion. San Diego, CA. Apr 1-5. Abst #AGFD 32.

Ide N, Ryu K, et al. 2001. 7th International Symposium on the Maillard Reaction. Kumamoto, Japan. Oct 29-Nov 1, p. 18.

Ide N, Ichikawa M, et al. 2002. In: International Congress Series 1245. The Maillard Reaction in Food Chemistry and Medical Science: Update for the Postgenomic Era. Elsevier Science, B.V., pp. 449-50.

Ide et al. reported that they found four of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids (MTCCs) as antioxidants in AGE. Interestingly, the concentrations of these compounds in AGE were shown to increase in progression of the natural aging process. Antioxidant properties of these compounds were further studied using several *in vitro* assay systems. All of four MTCCs showed strong hydrogen peroxide activities, particularly (1S, 3S)-1-methyl-1,2,3,4-tetrahydro- β -carboline-1,3-dicarboxylic acid [(1S, 3S)-MTCdiC], which was the most potent hydrogen peroxide scavenger and the activity was stronger than ascorbic acid. To elucidate the mechanism, data suggests that the metabolite of (1S, 3S)-MTCdiC may function as an electron donor and scavenge hydrogen peroxide. MTCCs also inhibited the peroxidation of linoleic acid caused by incubating with 2,2'-azobis(2-amidinopropane) hydrochloride [AAPH] at 37°C. Macrophages were incubated with lipopolysaccharides (LPS) at 37°C and 5% CO_2 for 20 hours and the release of nitric oxide (NO) metabolites were measured using a spectrophotometer. LPS significantly increased the release of NO metabolites from macrophages. Among four MTCCs identified in AGE, only dicarboxylates (1S, 3S) and (1R, 3S)-MTCdiC, significantly inhibited the release at low concentrations, suggesting that MTCCs, which are formed during the natural aging process, are potent antioxidants in AGE and that AGE would be useful for prevention of disorders associated with oxidative stress.

Yoshida J, Ide N, et al. 2001. 6th Annual Meeting of Japanese Society of Food Factors (JsoFF). Kobe, Japan. Nov 29-30, p. 22.

Four new antioxidants identified in AGE were reported. These compounds, 1,2,3,4-tetrahydro- β -carboline derivatives, showed strong scavenging activities. Among these compounds, (1S, 3S)-1-methyl-1,2,3,4-tetrahydro- β -carboline—1,3-dicarboxylic acid was found to be stronger than ascorbic acid. Chemical analytical data indicates that these four compounds were not detected in raw garlic, but their presence was increased during the natural aging process. These four new compounds may contribute to the antioxidant activities of AGE.

Ichikawa M, Ryu K, et al .2002. BioFactors. 16(3-4):57-72.

Ichikawa M, Ryu K, et al. 2004. Ch. 28. In: ACS Series 871. Nutraceutical Beverages: Chemistry, Nutrition and Health Effects, pp. 380-440.

Ichikawa M, Yoshida J, et al. 2006. J Nutr. 136(3 Suppl):726S-31S.

In a study that used liquid chromatography mass spectrometry (LC-MS), four tetrahydro-β-carboline derivatives were found to have strong hydrogen peroxide scavenging activities. These compounds found in AGE were shown to increase during the aging process and were not detected in raw garlic. This study suggests that these compounds are potent antioxidants and may play an important role in preventing disorders that are associated with oxidative stress.

Ichikawa M, Ryu K, et al. 2002. BioFactors. 16(3-4):57-72.

Ichikawa M, Ryu K, et al. 2004. Ch. 28. In: ACS Series 871. Nutraceutical Beverages: Chemistry, Nutrition and Health Effects, pp. 380-404.

Ichikawa M, Yoshida J, et al. 2006. J Nutr. 136(3 Suppl):726S-31S.

Tetrahydro-β-carboline derivatives (THβCs) hold a biological significance due to their antioxidant effects, anti-platelet aggregation and neuromodulations, such as inhibiting monoamine oxidase (MAO), biogenic amine (serotonin) uptake/release and benzodiazepine receptor binding. In the present study, four THβCs were identified in AGE, which is significant in demonstrating its antioxidant ability as a free radical scavenger.

Four alkaloids 1,2,3,4-tetrahydro-β-carboline derivatives (THβCs) were fractionated and identified in AGE: 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids (MTCC) and 1-methyl-1,2,3,4-tetrahydro-β-carboline-1, 3-dicarboxylic acid (MTCdiC) in both diastereoisomers using a hydrogen peroxide scavenging assay and were determined to have protective antioxidant effects.

Medina-Campos ON, Barrera D, et al. 2007. Food Chem Toxicol. 45(10):2030-9.

It was found that *S*-allyl cysteine (SAC) was able to scavenge concentration-dependently all the species assayed superoxide anion O_2^-, hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), and peroxynitrite anion ($ONOO^-$). When the ability of SAC to scavenge these species was compared to those of the reference compounds it was found that the efficacy of SAC (a) to

scavenge O_2^-, H_2O_2, OH^-, and $ONOO^-$ was lower, (b) to scavenge HOCl was similar, and (c) to scavenge $^1O_2$ was higher. In addition, it was found that SAC was able to prevent potassium dichromate ($K_2Cr_2O_7$)-induced toxicity in renal epithelial (LLC-PK_1) cells in culture.

Arguello-Garcia R, Medina-Campos ON, et al. 2010. J Agric Food Chem. 58:11226-33.

The hypochlorous acid (HOCl) scavenging capacities of 10 garlic compound containing modifications in the thioallyl group were determined. This scavenging activity was enhanced by increasing the number of sulfur (S) atoms or by the alanyl group and decreased in the absence of the C=C bond or in the presence of a sulfoxide group in the thioallyl group. *S*-allyl cysteine (SAC) and its corresponding sulfoxide alliin, showed the highest and lowest hypochlorous acid (HOCl)-scavenging capacities, respectively.

Morihara N, Hayama M,et al. 2011. Plant Foods Hum Nutr. 66(1):17-21.

In hypoxanthine-xanthine oxidase system, electron spin resonance showed that AGE scavenged superoxide radicals in a dose-dependent manner up to 54%. The half maximal effective concentration (EC_{50}) value of AGE for the superoxide radical scavenging effect was 0.80 mg/ml. *N*-α-(1-deoxy-D-fructos-1-yl)-L-arginine (25.9%) and (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid (20.8%), also exerted superoxide scavenging effects. Phorbol 12-myristate 13-acetate-activated human neutrophils produced superoxide radical of 56.6 ± 9.27 nmol/min/10^7 cells. AGE (3 mg/ml) significantly inhibited superoxide production in comparison to control.

Maldonado PD, Alvarez-Idaboy JR, et al. 2011. J Phys Chem B. 115(45):13408-17.

S-allyl cysteine (SAC) was able to scavenge hydroxyl radical (•OH) and peroxyl radical (ROO•), in a concentration-dependent way. Such activity was significantly ameliorated when the allyl group was replaced by benzyl (*S*-benzylcysteine [SBC]) or propyl (*S*-propylcysteine [SPC]) groups. It was shown for the first time that SAC is able to scavenge ROO•.

Sun YE, Wang WD. 2016. Cell Miol Biol (Noisy-le-grand). 62(7):85-9.

S-allyl-L-cysteine (SAC) was separated and identified from Allium sativum, and was reacted with 1-pyrenemethanol to obtain pyrene-labelled SAC (Py-SAC). The activity of Py-SAC and Vitamin C (VC) with oxygen radical absorbance capacity (ORAC) as index, the concentrations of Py-SAC and VC were 58.43 mg/L and 5.72 mg/L respectively to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 8.16 mg/L and 1.67 mg/L to scavenge •OH respectively. Compared with VC, the clearance rates of Py-SAC to scavenge DPPH were much higher, Py-SAC could inhibit hydroxyl radical.

Superior Antioxidant Activity Compared to Other Forms of Garlic

Imai J, Ide N, et al. 1994. Planta Med. 60(5):417-20.

AGE inhibited the emission of low level chemiluminescence and the early formation of thiobarbituric acid reactive substances (TBA-RS), whereas water extracts of raw and heat-treated garlic enhanced such emissions. *S*-allyl cysteine (SAC) and *S*-allyl mercaptocysteine (SAMC) in AGE also showed radical scavenging activity. Imai et al. suggested that AGE and its constituents have antioxidative efficacy whereas raw garlic and other preparations tend to stimulate oxidation.

Ichikawa M, Yoshida J, et al. 2006. J Nutr. 136(3 Suppl):726S-31S.

Using liquid chromatography-mass spectrometry (LC-MS), four types of 1,2,3,4-tetrahydro-β-carboline derivatives (THβCs) were isolated and identified in AGE and not in raw or other processed garlic preparations. These compounds show potent antioxidant properties in *in vitro* systems using the hydrogen peroxide scavenging assay. In particular, (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-1, 3-dicarboxylic acid or (1S, 3S)-MTCdiC is a more powerful hydrogen peroxide scavenger than ascorbic acid. This data suggests that AGE may be useful for the prevention of diseases associated with oxidative damage.

Amagase H, Petesch B, et al. 2001. J Nutr. 131(3 Suppl):955S-62S.

Garlic exhibits hypolipidemic, anti-platelet and pro-circulatory effects. In addition to these beneficial effects, AGE possesses hepato-protective, immune-enhancing, anti-cancer and chemopreventive activities. Furthermore, AGE exhibits antioxidative activities whereas other garlic preparations may stimulate oxidation. A wide variety of components in garlic work synergistically to provide various health benefits. Due to the complex chemistry in garlic, variations in processing yield quite different preparations. These additional biological effects seen in AGE may be due to converted components such as *S*-allyl cysteine (SAC) formed in the extraction process.

Ichikawa M, Ryu K, et al. 2002. BioFactors. 16(3-4):57-72.

Using liquid chromatography-mass spectrometry (LC-MS), four tetrahydro-β-carboline derivatives (THβCs) were found to have strong hydrogen peroxide scavenging activities. These compounds found in AGE were shown to increase during the aging process and were not detected in raw garlic. This study suggests that these compounds in AGE are potent antioxidants and may play an important role in preventing disorders that are associated with oxidative stress.

Ide N, Ryu K, et al. 2002. In:

Several *in vitro* assay systems and high-performance liquid chromatography (HPLC) were used to

International Congress Series 1245. The Maillard Reaction in Food Chemistry and Medical Science: Update for the Postgenomic Era. Elsevier Science B.V., pp. 447-8.

determine the antioxidant effects of fructosyl arginine (Fru-Arg), a compound in AGE. The study reported that Fru-Arg forms and is increased during the aging process and plays an important role as an antioxidant.

Ichikawa M, Ryu K, et al. 2003. Agric Food Chem. 51(25):7313-7.

New antioxidative compounds were found in the garlic skin. These compounds are also found in AGE. Six phenylpropanoids were identified. Determination and assay of these chemical compounds have been done by state-of-the-art chemical analysis, such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), etc.

Ide N, Ichikawa M, et al. 2003. Ch. 22. In: ACS Series 851. Food Factors in Health Promotion and Disease Prevention. American Chemical Society, pp. 250-63.

Liquid chromatography-mass spectrometry (LC-MS) was used to determine the antioxidant effects of four four tetrahydro-β-carboline derivatives (THβCs). These compounds were found to increase during the aging process and may be an important antioxidant in AGE.

Matsutoto T, Stark TD, et al. 2013. J Agric Food Chem. 61(12):3059-67.

In addition to the four tetrahydro-β-carboline derivatives (THβCs) that were previously reported, LC-MS/MS, LC-TOF-MS and 1D/2D-NMR experiments led to the identification of coniferyl alcohol and its dilignols (-)-(2R,3S)-dihydrodehydrodiconiferyl alcohol, (+)-(2S,3R)-dehydrodiconiferul alcohol, erthro-guaiacylglycerol-β-O-4'coniferyl ether, and threo-guaiacylglycerol-β-O-4'coniferyl ether as the major antioxidants in AGE. The purified individual compounds showed high antioxidant activity, with EC50 values of 9.7-11.8 μM (hydrogen peroxide scavenging or HPS assay) and 2.60-3.65 μmol TE/μmol (oxygen radical absorbance capacity or ORAC assay), respectively.

Cardioprotective Antioxidant Effects

—Protected Vascular System and Red Blood Cells from Oxidant Injury

Lin RI. 1990. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. Aug 28-30, p. 22.

When t-butylhydroperoxide, a free radical generator and oxidant, is used to oxidize red blood cells (RBC) resulting in the rupture of the cells and darkening of hemoglobin. AGE added to the RBC suspension prior to the addition of the oxidant minimizes such injuries. On the other hand, fresh garlic extract and raw garlic powder products enhanced the oxidative effects of this oxidant.

Yamasaki T, Lin L, et al. 1994. Phytother Res. 8:408-12.

AGE and *S*-allyl cysteine (SAC) can protect cells which line the blood vessels of the lungs from oxidant injury. AGE and SAC in this *in vitro* test, protected pulmonary endothelial cells from hydrogen peroxide (H_2O_2)-induced oxidant injury. Pretreatment of cells overnight with AGE (2-4 mg/ml) or SAC (4 mg/ml) significantly reversed the loss of cell viability, inhibited lactate dehydrogenase (LDH) release and lipid peroxidation induced by H_2O_2. Yamasaki et al. suggested that these compounds may be effective in hampering the aging process and for the prevention of atherosclerosis.

Morihara N, Ide N, et al. 2005. Redox Report. 10(3):159-65.

When AGE is combined with a suspension of erythrocytes, it decreased peroxynitrite-induced hemolysis in a concentration-dependent manner. *S*-allyl cysteine (SAC), found in AGE, was also found to decrease hemolysis. Since peroxynitrite is a strong oxidant, it has been shown to cause vascular or tissue damage. Therefore, AGE and its constituents may be helpful for preventing cardiovascular diseases and may help prevent the damage of membranes in erythrocytes.

—Protection in Myocardial Infarction

Padmanabhan M, Mainzen Prince PS. 2007. Life Sci. 80(10):972-8.

In models who suffered myocardial infarction due to prolonged myocardial ischemia showed improvement in mitochondrial enzyme activities when pretreated with *S*-allyl cysteine (SAC). Padmanabhan et al. suggest that this is due to SAC antioxidant qualities.

Wang Q, Qang XL, et al. 2010. Antioxid Redox Signal. 12(10):1155-65.

S-allyl cysteine (SAC), *S*-propyl-L-cysteine (SPC) and *S*-propargyl-L-cysteine (SPRC) were found to preserve superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in an acute myocardial infarction (MI) models and also tissue glutathione (GSH) levels while reducing the formation of the lipid peroxidation product malonaldehyde (MDA) in ventricular tissues. This study provides novel evidence that SAC, SPC and SPRC have cardioprotective effects in MI by reducing the deleterious effects of oxidative stress by modulating the endogenous levels of hydrogen sulfide (H_2S) and preserving the activities of antioxidant defensive enzymes.

—Improved Endothelial Dysfunction

Tsuneyoshi T, Kanamori Y, et al. 2015. Biochem Biophys Res Commun. 465(3):408-13.

Antioxidant lignan compounds isolated from AGE (+)-(2S,3R)-dehydroconiferyl alcohol (DDC) and (-)-(2R,3S)-dihydroehydroiconiferyl alcohol (DDDC) were shown to suppress the adhesion of THP-1 monocyte to human umbilical vein endothelial cells (HUVECs) which was activated by lipopolysaccharide (LPS) or advanced glycation endproducts (AGEs-BSA) and inhibited vascular cell adhesion molecule 1 (VCAM-1) expression induced by LPS or AGEs-BSA, although DDDC was less effective than DDC. In addition, the inhibitory effect of DDC on VCAM-1 expression involved suppressing JNK/c-Jun pathway. These results suggest that DDC may improve endothelial dysfunction.

—Decreased Cardiotoxicity of Anti-Cancer Drug Doxorubicin

Kojima R, Toyama Y, et al. 1994. Nutr Cancer. 22(2):163-73.

Doxorubicin (DOX), a cardiotoxic drug used to treat cancer, causes vacuolization in muscle cells, disruption of myofibrils, swollen mitochondria and lipid peroxidation in heart tissues. It was found that AGE taken in conjunction with DOX showed significantly less lipid peroxidation and no significant pathological lesions in the hearts of models compared to those treated with DOX alone.

Kojima R, Toyama Y, et al. 1994. American Heart Association's 67th Scientific Session. Dallas, TX. Nov 14-17. Abst # 032282

Models receiving AGE in conjunction with doxorubicin (DOX) had better weights, survival and echocardiograms (ECG) than those receiving DOX alone. Though the hearts treated with DOX alone exhibited significant pathology, those treated with DOX and AGE were almost normal.

Ohnishi S, Kojinra R. 1997. Ch. 12. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 105-15.

Both AGE liquid (0.05/20g body weight, i.p.) and Kyolic AGE tablets (0.01-0.02g/20g body weight, p.o.), given 6 times weekly to models, protected the heart against the cardiotoxicity of doxorubicin (DOX) (1.5mg/kg body weight given 3 times a week for 40 days). AGE assisted in maintaining a normal QRS width. Furthermore, AGE inhibited lipid peroxidation as seen by a decrease in thiobarbituric acid reactive substances (TBA-RS). DOX alone increased TBA-RS in heart tissue by 76% whereas AGE liquid reduced TBA-RS to 17%. AGE also ameliorated the decrease in body weight and survival rate caused by DOX administration.

Awazu S, Horie T. 1997. Ch. 14. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 131-8.

Diallyl pentasulfide, a constituent in AGE, inhibited the production of oxidants by doxorubicin (DOX), a cancer drug. It completely inhibited both thiobarbituric acid reactive substances (TBA-RS) and chemiluminescence in heart tissue incubated with DOX and reduced nicotinamide adenine dinucleotide (NADH).

Mostafa MG, Mima T, et al. 2000. Planta Med. 66(2):148-51.

Doxorubicin (DOX), a potent anticancer drug, is effective against a wide range of human cancers. However, the clinical uses of DOX have been limited due to its serious cardiotoxic adverse effects, which are likely the result of generation of free radicals and lipid peroxidation. *S*-allylcysteine (SAC), an antioxidative compound from AGE, was found to significantly reduce DOX-induced mortality, heart and liver damage. It was suggested that SAC research may ultimately lead to a resolution to the adverse effects of DOX treatment in cancer chemotherapy.

Demirkaya E, Avci A, et al. 2009. Can J Physiol Pharmacol. 87(8):633-40.

Models received AGE for 6 weeks in addition to their standard diet and treated with cumulative intraperitoneal (i.p.) injections of high-dose of doxorubicin (DXR) or low-dose DXR for 4 weeks. Electron microscope of heart tissue changes clearly demonstrated the protective effects of AGE.

Alkreathy H, Damanhouri ZA, et al. 2010. Food Chem Toxicol. 48(3):951-6.

A single-dose of doxorubicin (DOX) caused increased both serum cardiac enzymes lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK) activities and a significant increase malonyldialdehyde (MDA) in plasma. However, pretreatment of models with AGE (250 mg/kg) for 27 days before DOX therapy, reduced the activity of both enzymes, and significantly decreased of MDA production in plasma. Total antioxidant activity was increased after AGE administration and ameliorated the effect of DOX administration on cardiac tissue; cardiomyocytes looked more or less similar to those of control.

—Prevented Oxidation of LDL Cholesterol in Clinical Studies

Munday JS, James K, et al. 1999. Atherosclerosis. 143(3):399-404.

AGE given at 2.4g for 7 days made the low-density lipoproteins (LDL) of subjects significantly more resistant to oxidation by copper (Cu^{2+}). Lag time for oxidation increased 27% (86.5 to 119 minutes, $p<0.01$). Six grams of raw garlic, on the other hand, was ineffective.

Lau BHS. 2006. J Nutr. 136(3 Suppl):765S-8S.

A double-blind, placebo-controlled, cross-over study found that ingestion of AGE significantly increased the resistance of plasma low-density lipoproteins (LDL) to oxidation.

—Prevented Oxidation of LDL Cholesterol in Pre-Clinical Studies

Ide N, Lau BHS, et al. 1997. Experimental Biology. New Orleans, LA. Apr 6-9. FASEB J. 11(3):A122. Abst #713.

Ide N, Nelson AB, et al. 1997. Planta Med. 63(3):263-4.

AGE and its various constituents were found to inhibit copper-induced peroxidation of low-density lipoproteins (LDL) cholesterol in a concentration-dependent manner. Lipid oxidation was determined by measuring thiobarbituric acid reactive substances (TBA-RS). When AGE was preincubated with pulmonary artery endothelial cells, cell damage caused by oxidized LDL was prevented as indicated by the prevention of lactate dehydrogenase (LDH) release, loss of cell viability and TBA-RS formation. Not does AGE only prevent the oxidation of LDL, but also prevented oxidized LDL from damaging membranes, oxidizing lipids and damaging cells which may contribute to the initiation and/or progression of cardiovascular disease.

Ide N, Lau BHS. 2001. J Nutr. 131(3 Supp):1020S-6S.

The effects of AGE and its major compound *S*-allyl cysteine (SAC) on oxidized low-density lipoproteins (Ox-LDL)-induced injury on endothelial cells (EC). Lactate dehydrogenase (LDH) release, an index of membrane damage, methiazol tetrazoium (MTT) assay for cell viability and thiobarbituric acid reactive substances (TBA-RS) indicating lipid peroxidation, were measured. Ox-LDL caused an increase of LDH release, loss of cell viability and TBA-RS formation. AGE or SAC prevented all these changes. Pretreatment of EC with SAC inhibited nuclear factor-κB (NF-κB) activation while AGE and SAC protected EC from Ox-LDL-induced injury preventing intracellular glutathione (GSH) depletion in EC and by releasing peroxides from EC and macrophages (MΦ). SAC also inhibited hydrogen peroxide (H_2O_2) or tumor necrosis factor-alpha (TNF-α)-induced NF-κB activation. This data suggest that AGE and SAC may be useful for the prevention of atherosclerosis.

Lau BHS. 2001. J Nutr. 131(3 Suppl):985S-8S.

Short-term supplemention of garlic in models demonstrated an increased resistance of low-density lipoproteins (LDL) oxidation suggesting that suppressed LDL oxidation may be one of the mechanisms accounted for the anti-atherosclerotic properties of garlic.

Ho S, Ide N, et al. 2001. Phytomedicine. 8(1):39-46.

S-allyl cysteine (SAC) prevented the copper-induced oxidation of low-density lipoprotein (LDL) cholesterol (ox-LDL) and further damage caused by Ox-LDL in J774 macrophages and another sensitive cell line. Nuclear factor κB (NF-κB) is activated by oxidants, such as hydrogen peroxide and by tumor necrosis factor-α (TNF-α). NF-κB in immune T cells is involved in immune and inflammatory reactions. When incubated together, SAC was shown to inhibit the activiation of NF-κB by both TNF-α and by hydrogen peroxide. Since both Ox-LDL and NF-κB are involved in atherogenesis, SAC may act via antioxidant mechanism to inhibit the atherogenic process.

Dillon SA, Burmi RS, et al. 2003. Life Sci. 72(14):1583-94.

Human low-density lipoproteins (LDL) was isolated and challenged with a range of oxidants either in the presence of AGE or its diethyl ether extract. Results of this study indicate AGE's ability to inhibit the *in vitro* oxidation of LDL by scavenging superoxide and inhibiting the formation of lipid peroxides. AGE also reduced LDL oxidation by chelating copper ions. Thus, AGE may have a role to play in preventing the development and progression of atherosclerotic disease.

—Prevented Damage to Cells Caused by Oxidized LDL Cholesterol

Ide N, Lau BHS. 1997. J Pharm Pharmacol. 49(9):908-11.

AGE could prevent damage to cell membranes caused by oxidized low-density lipoproteins (Ox-LDL) cholesterol. Lactate dehydrogenase (LDH) is an enzyme found inside of cells that leak out into the culture medium when cell membranes are damaged. A higher level of LDH in the solution indicates a greater level of membrane damage. When Ox-LDL was added to endothelial cells, it caused about 30% of the LDH to be release. However, AGE caused only 18-22% of LDH to be released, inhibiting 35-51% of the membrane damage caused by Ox-LDL.

Steiner M, Lin RS. 1998. J Cardiovasc Pharmacol. 31(6):904-8.

In a 10-month placebo-controlled study in men with high cholesterol that received supplementation of 7.2g of AGE elicited a trend toward reduced oxidation of cholesterol. Oxidized cholesterol is more likely to adhere to the lining of the veins and cause scarring than cholesterol that has not been oxidized.

Ide N, Lau BHS. 1999. Phytomedicine. 6(2):125-31.

Pretreatment of pulmonary endothelial cells with AGE prevented cell damage as measured by a decrease in lactate dehydrogenase (LDH) and depletion of the glutathione (GSH) caused by oxidized low-density lipoproteins (Ox-LDL). AGE also reduced Ox-LDL peroxides or free radicals generated from Ox-LDL.

—Ameliorated Damaging Effects of Nitric Oxide

Ide N, Lau BHS. 1999. Phytomedicine. 6(2):125-31.

AGE inhibited nitric oxide (NO) and peroxide production in J774 macrophage immune cells and scavenged the free radical hydrogen peroxide in a dose-dependent manner.

Attenuated Ischemic Brain Damage

Numagami Y, Sato S, et al. 1996. Neurochem Int. 29(2):135-43.

When *S*-allyl cysteine (SAC) was administered 30 minutes prior to ischemic insult, there was a significant decrease in ischemic damage. This was indicated by decreased water (swelling of the brain) in the middle cerebral artery occlusion model. In a global ischemia model, SAC decreased the amount of reactive oxygen species generated due to ischemia.

Numagami Y, Ohnishi S. 2001. J Nutr. 131(3 Suppl):1100S-5S.

In a middle cerebral artery occlusion model, pre-ischemic administration of *S*-allyl cysteine (SAC) improved (i) motor performance and (ii) memory impairment, (iii) reduced water contents and (iv) infarct size. In a transient global ischemia model, (i) the production of free radicals as studied by an electron paramagnetic resonance spectroscopy (EPR) was biphasic; the first peak occurring at 5 minutes and second peak at 20 minutes after reperfusion. SAC did not attenuate the first peak but did on the second peak. (ii) The lipid peroxidation as estimated by thiobarbituric acid reactive substances (TBA-RS) increased significantly at 20 minutes after reperfusion. SAC decreased TBA-RS to the levels found without ischemia. These results suggest that SAC would have beneficial effects in brain ischemia and that the major protective mechanism may be inhibition of free radical-mediated lipid peroxidation.

Kim KM, Lee JC, et al. 2006. Free Radic Res. 40(8):827-35.

S-allyl cysteine (SAC) decreased the size of infarction after transient or global ischemic insults. While it did not alter the *N*-methyl-D-aspartate excitotoxicity, SAC significantly scavenged the endogenously or exogenously produced peroxynitrite ($ONOO^-$) and reduced $ONOO^-$ cytotoxicity. SAC also inhibited the activity of extracellular signal-regulated kinase (ERK) increased in cultured neurons exposed to oxygen-glucose deprivation. The results indicate that SAC exerts its neuroprotective effect by scavenging $ONOO^-$ and inhibiting the ERK signaling pathway activated during initial hypoxic/ischemic insults.

Garcia E, Limon D, et al. 2008. Free Radic Res. 42(10):892-902.

In 3 models exerting striatal toxicity, *S*-allyl cysteine (SAC) was shown to prevent lipid peroxidation (LP) and mitochrondrial dysfunction (MD) in synaptosomal fractions from 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridinium-treated models, but without complete restoration of dopamine levels in the first model. In the second model, SAC prevented LP and MD in synaptosomes from models infused with 6-hydroxydopamine into the *substantia nigra pars compacta*, but again, without total reversion of depleted dopamine levels. In the third model, SAC prevented MD in synaptosomes injected with 3-nitropropionic acid, but failed to prevent LP. SAC also prevented the aberrant motor activity patterns evoked by the three toxins. Altogether, these results suggest that the antioxidant properties of SAC are responsible for partial or total preservation of neurochemical, biochemical and behavioral markers, indicating that pro-oxidant reactions underlie the neurotoxicity in these models.

Atif F, Yousuf S, et al. 2009. Brain Res. 1265:128-37.

In a transient middle cerebral artery occlusion (MCAO) model with ischemia/reperfusion (I/R)-induced mitochondrial dysfunctions, *S*-allyl cysteine (SAC) significantly restored adenosine triphosphate (ATP) content and the activity of mitochondrial respiratory complexes, which were severely altered. A marked decrease in calcium swelling and decrease in cytochrome c release were observed as a result of SAC treatment. SAC also restored the status of mitochondrial glutathione (GSH) and glucose 6-phosphate dehydrogenase (G6-PD), significantly decreased mitochondrial lipid peroxidation (LPO), protein carbonyl (PC) and hydrogen peroxide (H_2O_2) content, significantly improved neurological deficits and significantly reduced brain edema.

Aguilera P, Chanez-Cardenas ME, et al. 2009. Phytomedicine. 17(3-4):241-7.

Models subjected to middle cerebral artery occlusion (MCAO) for 2 hours were treated with 1.2 ml/kg body weight (i.p.) AGE 30 minutes before, at the beginning of reperfusion (0R), or 1 hour after. The 0R treatment significantly reduced the size of the infarct area after 2 hours of reperfusion. The protective 0R treatment with AGE prevented the increase in nitrotyrosine and the decrease in total superoxide dismutase, glutathione peroxidase and extracellular superoxide dismutase activities induced by MCAO. This indicates that AGE delays the effects of ischemia/reperfusion-induced neuronal injury.

Colin-Gonzales AL, Ortiz-Plata A, et al. 2011. Plant Foods Hum Nutr. 66(4):348-54.

Models were subjected to 1 hour of ischemia plus 24 hours of reperfusion while AGE (1.2 ml/kg weight, i.p.) was administered at onset of reperfusion. AGE treatment diminished the neurological alterations (61.6%), the infarct area (54.8%) and the histological damage (37.7%) induced by cerebral ischemia. AGE administration attenuated the increase in 8-hydroxy-2-deoxyguanosine (8-OhdG) levels (77.8%) and tumor necrosis factor α (TNFα) levels (76.6%) and in cyclooxygenase-2 (COX-2) protein levels (73.6%)

and activity (30.7%) induced after 1 hour of ischemia plus 24 hours of reperfusion.

Ashafaq M, Khan MM, et al. 2012. Nutr Res. 32(2):133-43.

Models were subjected to middle cerebral artery occlusion (MCAO) for 2 hours and 22-hour reperfusion. *S*-allyl cysteine (SAC) was administered intraperitoneally 30 minutes before the onset of ischemia and after the ischemia at the interval of 0, 6 and 12 hours. After 24 hours, SAC treatment significantly reduced ischemic lesion volume, improved neurological deficits, combated oxidative loads and suppressed neuronal loss. Behavioral and biochemical alterations observed after MCAO were further associated with an increase in glial fibrillary acidic protein and inducible nitric oxide expression and were markedly inhibited by SAC treatment.

Reduced Depletion of Circulatory Antioxidants Caused by a Cancer-Causing Agent

Balasenthil S, Nagini S. 2000. J Biochem Mol Biol. 4:35-9.

7,12-dimethylbenz(a)anthracene (DMBA) significantly depletes circulating antioxidants such as ascorbic acid and vitamin E, reduces glutathione and glutathione peroxidase and enhances lipid peroxidation in the circulation of tumor-bearing models. Administration of *S*-allyl cysteine (SAC), a constituent in AGE, significantly decreased lipid peroxidation and enhanced the levels of antioxidants when exposed to DMBA-induced oxidative stress.

Protected Against Oxidative Stress and Renal Changes from an Anti-Cancer Drug

Nasr AY, Saleh HA. 2014. Cancer Cell Int. 14(1):92.

Cisplatin (CP) is an effective anticancer drug but causes many side effects. Models were divided into 4 equal groups: control, AGE (250 mg/kg, once oral dose/21 days), CP-treated, combined AGE and CP-treated were used. Hemorrhage, glomerular atrophy, inflammatory cell infiltration, tubular necrosis and degeneration were observed in CP-treated models. Also, a significant ($P<0.001$) reduction in superoxide dismutase (SOD) and catalase (CAT) activities, reduced glutathione (GSH) levels accompanied with a significant increase in serum levels of kidney biomarkers and malondialdehyde (MDA) were determined in CP-treated models compared to control group. However, most of CP-induced histomorphological, ultrastructural and biochemical changes were improved in models pretreated with AGE.

Youssef Nasr A, Al Shahat Ibrahim A. 2015. Microsc Res Tech. 78(6):452-61.

Superoxide dismutase and catalase activities and glutathione levels were significantly decreased and malondialdehyde level was significantly increased in cisplatin (CP)-induced nephrotoxicity in models compared to AGE + CP-treated group. A remarkable improvement in the histopathological and ultrastructural changed induced by CP in renal tissues was also observed in AGE + CP-treated group.

Antioxidative Effect: Review

Ide N. 1999. 30th Chugoku/Shikoku Branch Meeting on the Chemical Society of Japan. Hiroshima, Japan. Apr 23, pp. 25-9.

AGE was shown to directly scavenge the reactive oxygen species (ROS), superoxide radical and hydrogen peroxide and inhibit lipid peroxidation. AGE also demonstrated the ability to inhibit low-density lipoprotein oxidation (Ox-LDL), protect endothelial cells from Ox-LDL-induced cell injury by removing excess ROS and inhibit intracellular glutathione (GSH) depletion.

Moriguchi T, Ide N, et al. 2001. Vitamin (Tokyo). 75:515-6.

In previous studies, AGE has been shown to inhibit the formation of lipid peroxidation and oxidized low-density lipoproteins (Ox-LDL), protect endothelial cells from oxidative stress *in vitro* and protect red blood cells through antioxidant effects *in vivo*. AGE is produced through the natural aging process of garlic, forming unique biological compounds during this time. Since these unique compounds have been reported to show strong antioxidant effects, they may play an important role in the antioxidant effects of AGE.

Banerjee SK, Mukherjee PK, et al. 2003. Phytother Res. 17(2):97-106.

Numerous studies show AGE's potential as an antioxidant. Many scientific research studies support that AGE may help protect against aging, radiation, chemical exposure, and many oxidant-induced disease conditions.

Colin-Gonzalez AL, Santana RA, et al. 2012. Oxid Med Cell Longev. 907162.

Different antioxidant mechanisms (scavenging of free radicals and pro-oxidant species, induction of antioxidant enzymes, activation of nuclear factor-E2 related factor 2 [Nrf2 factor], inhibition of pro-oxidant enzymes and chelating effects) involved in the protective actions of AGE and SAC were reviewed. In addition, the ability of *S*-allyl cysteine (SAC) to activate Nrf2 factor—a master regulator of the cellular redox state is highlighted. Original data showing the ability of SAC to activate Nrf2 factor in cerebral cortex is included.

Capasso A. 2013. Molecules. 18(1):690-700.

AGE contains stable water-soluble organosulfur compounds that have been found to influence an increasing number of molecular mechanisms in carcinogenesis, including DNA adduct formation, scavenging of free radicals, mutagenesis, cell proliferation and differentiation, and angiogenesis.

Anti-Stress and Anti-Fatigue Effects

Improved Recovery from Subjective Symptoms from Various Internal Diseases in Clinical Studies

Hasegawa Y, Kikuchi N, et al. 1983. Shinyaku to Rinsho (Jpn J New Remedies Clin). 32:365-76.

One hundred and thirty subjects experienced improvements in various subjective symptoms including systemic, neuromuscular, respiratory, cardiovascular and digestive complaints following the intake of Kyoleopin® (KLE). Improvements in fatigue, weakness and constipation were also noted.

Okada K, Miyagaki H, et al. 1983. Kiso to Rinsho (Preclin Clin Reports). 17:2173-83.

In a clinical study, Kyoleopin® (KLE) was found to be moderately to highly effective in improving subjective symptoms in patients. Subjective symptoms included non-specific complaints such as fatigue, headache, heavy head, stiff shoulders, eye fatigue, etc. A trend toward stabilized blood pressure was also noted (i.e., increases for low blood pressure and decreases for high blood pressure).

Miyoshi A, Kasegawa Y, et al. 1984. Shinryou to Shinyaku (Treatment New Med). 21(10):1806-20.

Kyoleopin® (KLE) was given to more than 1,000 subjects suffering from unexplained complaints which often accompany internal diseases. Moderate and apparent improvement in fatigue, dizziness, debility, anorexia and headache was found in the majority of subjects.

Hasegawa Y, Kikuchi N, et al. 1984. Shinryou to Shinyaku (Treat New Med). 21(10):2021-35.

Of 132 subjects suffering from indefinite medical complaints, slight or better improvement was noted in 91% of patients taking Kyoleopin® (KLE) who exhibited fatigue, 90% who exhibited poor physical condition, 83% who exhibited stiff shoulders, 82% who exhibited lethargy and 81% who exhibited poor appetite. Total effectiveness (rated slightly effective or better) was 95% and the supplement was ineffective in only 7 cases. Some noteworthy findings included healthy weight gain, improved anemia, a beneficial effect on cholesterol metabolism and a tendency towards normalization of the liver enzyme glutamate oxaloacetate transaminase (GOT).

Kawashima H, Ochiai Y, et al. 1985. Shinryou to Shinyaku (Treatment New Med). 22(12):3012-24.

Kyoleopin® was administered to 39 patients with the cold and general fatigue. Significant improvements in chills, abdominal pain, general fatigue, general myalgia and physical disorders were observed. Patients also noted improvement in headaches, lumbago, joint and chest pain. Improved patellar reflex values provided an objective measurement to confirm the anti-fatigue effects of AGE preparation in this study.

Reduced Physiological Stress in Pre-Clinical Studies

Ushijima M, Sumioka I, et al. 1997. Phytother Res. 11:226-30.

Amagase H, Matsuura H, et al. 2000. Ch. 6. In: Phytochemicals and Pharmaceuticals. AOCS Press. Champaign, IL, pp. 62-78.

AGE was more effective than raw garlic juice (RGJ), heated garlic juice (HGJ) or powdered garlic (PG) at reducing both physiological and psychological stress in various stress tests. Models who were given AGE swam 82% (2 ml) and 90% (4 ml) of the time there were in water, whereas those who were not given garlic swam only 50% of the time. Except at a low dosage of raw garlic, only AGE significantly enhanced swimming time. Models given AGE were also found to run longer than those given a placebo (control), RGJ, HGJ or PG in a mechanical treadmill running test. Control ran for 929 seconds whereas those given AGE ran for 1611 seconds, almost twice as long.

Ishii S, Ushijima M, et al. 2006. Oyo Yakuri (Pharmacometrics). 71(3/4):101-9.

Ishii S, Nishihama T. 2007. 7th Annual Scientific Meeting of the Japanese Society of Anti-Aging Medicine. Jul 20-21. Abst #P096.

Kyoleopin Neo® (KLEN) attenuates the reduction of skin surface conductance (SSC), induced by immobilization stress and increases skin pigmentation induced by ultraviolet B (UVB) in models, suggesting that it may function as a useful agent for improvement of skin condition deterioration caused by various internal or external factors.

Reduced Physiological Stress in Clinical Studies

Yaguchi S, Tokoro K, et al. 2005. Shinyaku to Rinsho (New Drug Clin). 42(2):189-96.

Subjects with unexplained complaints and malaise were given 2 ml of Leopin Royal® (LER) daily for 4 weeks. Supplementation with LER reduced stress indexes and improved symptoms such as chills, stiffness in the shoulders, fatigue lassitude, decline of physical strength/willpower, headache, abnormal bowel movement and lumbago. The efficacy of LER was felt by 87.5% of subjects, indicating that LER is useful for various unexplained complaints and malaise.

Ishii S, Nishihama T, et al. 2006. Oyo Yakuri (Pharmacometrics). 70(3/4):97-105.

In a 4-week human trial, 1 ml of Kyoleopin Neo® (KLEN) was administered to subjects twice a day after meals in the morning and evening. Fatigue was evaluated at 0, 2 and 4 weeks after taking KLEN and using an Advanced Trail Making Test (ATMT); a tool used to measure the performance of brain function

associated with mental fatigue. KLEN decreased "brain-age" time-dependently and significantly. Demonstrating that KLEN may be useful for ameliorating daily fatigue or for preventing the accumulation of fatigue.

Womack CJ, Lawton DJ, et al. 2015. J Int Soc Sports Nutr. 12:23.

Healthy trained males were randomly assigned to ingest either 900 mg of AGE or placebo three hours before the exercise session (graded treadmill test to volitional exhaustion) in a double-blind, crossover fashion with a 14-day washout period. The maximum oxygen uptake (VO_2max) was greater for the garlic treatment group versus placebo (Placebo = 59.8 ± 6.7 ml • kg^{-1} • min^{-1}; Garlic = 61.4 ± 6.6 ml • kg^{-1} • min^{-1}).

Improved Recovery from Athletic Performance in Pre-Clinical Studies

Kasuga S, Morihara N, et al. 1998. Hokkaido J Sports Med Sci. 6:29-36.

The effect of AGE for endurance performance, succinate dehydrogenase (SDH) activity in the models' gastrocunemius muscle and soleus muscle and plasma nitric oxide (NO) metabolic concentrations increased significantly. The results showed that AGE would facilitate the skeletal muscle oxidative enzyme activity and contribute to enhancement of physical strength and endurance training.

Improved Recovery from Athletic Performance in Clinical Studies

Kohno M, Shirakura T, et al. 1976. Yakuri to Chiryo (Jpn J Pharmacol Ther). 4(3):700-8.

In a double-blind study, Kyoleopin® (KLE) was given to personnel of the Omiya Camp of the Japanese Land Self-Defense Force. Subjects taking KLE recovered from exhaustion faster and made fewer complaints of exhaustion and tiredness after manual labor than those taking placebo. Results were more notable in those aged 45-50 years than 25-35 years.

Kawashima H, Ochiai Y, et al. 1986. Kiso to Rinsho (Preclin Clin Reports). 20(16):8229-45.

AGE 2 ml twice a day given to 20 healthy male college students improved subjective and objective symptoms of fatigue following 22 days of intense physical training. AGE also improved patellar reflex and promoted serum levels of glutamate oxaloacetate transaminase (GOT), glutamic-pyruvic transminase (GPT), triglycerides and lactic acid, which favor relief from fatigue. These latter effects provided further objective measurements of the anti-fatigue effects of AGE. AGE was concluded to be an effective supplement for the prevention of and recovery from fatigue.

Kimoto R, Kambayashi I, et al. 2005. Hokkaido J Sports Med Sci. 10:17-26.

AGE's influence on the change of urinary 8-hydroxydeoxyguanosine (8-OhdG) content, which is thought to be a marker of oxidative stress, during daily regular and temporary intense exercise, was investigated. Twelve healthy males were divided into two groups: AGE group and control group. AGE group was given AGE for 2 weeks. Urinary 8-OhdG content was found to be significantly lower with the AGE group than with the control. Also, AGE significantly increased the sum total of oxygen uptake during intense exercise.

Womack CJ, Lawton DJ, et al. 2015. J Int Soc Sports Nutr. 12:23.

Healthy trained males were randomly assigned to ingest either 900 mg of AGE or placebo three hours before the exercise session (graded treadmill test to volitional exhaustion) in a double-blind, crossover fashion with a 14-day washout period. The maximum oxygen uptake (VO_2max) was greater for the garlic treatment group versus placebo (Placebo = 59.8 ± 6.7 ml • kg^{-1} • min^{-1}; Garlic = 61.4 ± 6.6 ml • kg^{-1} • min^{-1}).

Improved Recovery from Chemical and Physical Stress

Takasugi N, Kotto K, et al. 1984. Oyo Yakuri (Appl Pharmacol). 28(6):991-1002.

AGE was shown to accelerate recovery from fatigue induced by exposure to 4 hour oscillation movement, chronic rope climbing and prolonged cold stimulus (4°C). Furthermore, AGE improved learning test results exposed to 40% alcohol administration and accelerated recovery from alcohol-induced disruption of motor activity. AGE was also shown to accelerate the removal of blood alcohol in alcohol-treated models.

Ushijima M, Mizuno I, et al. 2005. J Jpn Mibyou System Assoc. 11:111-6.

Ushijima M, Mizuno I, et al. 2005. 11th Annual Meeting of Jpn Mibyou System Assoc. Saitama, Japan. Jan 8-9. Abst #26.

The anti-stress effect of Leopin Royal® (LER) was evaluated using a model system. Physical and social stresses have been determined to lower momentum and the ability for antibody production. LER was able to improve this state in models. LER has also been shown to promote spermatogenesis. This data indicates that LER has positive effects on recovering from physical fatigue, modulating the immune system and promoting spermatogenesis, suggesting that LER may be useful for reducing symptoms induced by various stressors.

Improved Recovery from Oscillation Stress (Dizziness)

Takasugi N, Kira K, et al. 1986. Oyo Yakuri (Appl Pharmacol). 31(5):967-76.

AGE preparations given 10 minutes prior to and immediately after exercise prevented physical and mental disorders induced by acute oscillation stress.

Prevented Stress-Induced Hypertrophy of the Adrenal Gland and Hyperglycemia

Kasuga S, Ushijima M, et al. 1999. Nippon Yakurigaku Zasshi (Folia Pharmacol Jpn). 114:191-7.

Pretreatment with AGE (5 and 10 ml/kg, p.o.) significantly prevented adrenal hypertrophy, hyperglycemia and elevation of corticosterone, without altering insulin level, exposed to 2 days (16 hours/day) of immobilization stress.

Amagase H, Matsuura H, et al. 2000. Ch. 6. In: Phytochemicals and Pharmaceuticals. AOCS Press. Champaign, IL, pp. 62-78.

AGE (10 ml/kg) was reported to prevent stress-induced hypertrophy of the adrenal gland in models undergoing immobilization stress. Raw garlic juice, heated garlic juice and powdered garlic showed no protective effects.

Improved Stress-Induced Immunosuppression

Yokoyama K, Uda N, et al. 1986. Oyo Yakuri (Appl Pharmacol). 31(5):977-84.

AGE preparations restored stress-induced suppression of antibody production and atrophy of the spleen and provided anti-stress and anti-fatigue benefits.

Kyo E, Uda N, et al. 1999. Phytomedicine. 6(5):325-30.

AGE prevented the suppression of the immune system caused by psychological stress. After 4 days exposure to psychological stress, a significant decrease in spleen weight and spleen cells was observed compared non-stressed condition. AGE significantly prevented these decreases, as well as a reduction of hemolytic plaque-forming-cells and anti-sheep red blood cell (SRBC) antibody titer in serum caused by this psychological stress. AGE maintained natural killer (NK) immune cell activities almost to the level of non-stressed condition, whereas stressed models without AGE showed suppressed NK activity. It was concluded that psychological stress qualitatively and quantitatively impairs immune function, and that AGE prevents such damage.

Reduced Stress-Induced Activation of the Peripheral Sympathetic System

Kvetnansky R, Takasugi N, et al. 1990. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. Aug 28-30, p. 46.

AGE administered acutely or repeatedly for 14 days (30, 100 or 300 mg/kg, p.o.) reduced the stress-induced activation of the peripheral sympathetic system without affecting the adrenal medulla or pituitary-adrenocortical system. Administration of a 30 mg dose of AGE in unstressed models reduced norepinephrine (NE) and adrenocorticotropic hormone (ACTH) levels whereas high dosages only increased epinephrine levels. Repeated administration reduced NE even more. AGE in conjunction with immobilization stress reduced NE levels. Administration of a single 300 mg dose of AGE slightly reduced ACTH, corticosterone and plasma prolactin levels.

Inhibits Stress-Induced Peptic Ulcer Formation

Nagai K, Kumagai T, et al. 1972. Kiso to Rinsho (Preclin Clin Reports). 6:1536-8.

Kyoleopin® (KLE) orally administered at dosages of 2.0, 0.2 and 0.02 ml clearly prevented stress-induced ulcer formation. Nagai et al. attributed the anti-ulcer effects to strengthening of the gastric mucosa rather than to a decrease in aggravating agents such as gastric acid and pepsin.

Anti-Stress/Anti-Fatigue Effect: Review

Morihara N, Nishihama T, et al. 2007. Mol Nutr Food Res. 51(11):1329-34.

Garlic is also used for the treatment of fatigue, although the mechanism involved remains unclear. In model studies, garlic has been shown to promote exercise endurance. Differences in the methods of processing garlic result in differences in the intensity of its anti-fatigue effect, and the most favorable form of processing has been shown to be AGE. In human studies, it has been confirmed that garlic produces symptomatic improvements in persons with physical fatigue through a variety of actions. Current available data strongly suggests that garlic may be a promising anti-fatigue agent, and that further studies to elucidate its application are warranted.

Singh SV. 2001. J Nutr. 131(3 Suppl):1046S-8S.

Oral administration of diallyl disulfide (DADS) resulted in a dose-dependent and statistically significant inhibition of protein H-ras oncogene transformed tumor growth. The tumor growth inhibitory effect of DADS was apparent in terms of delay in the appearance of measurable tumors, tumor volume as well as tumor weight. DADS suppresses the growth of H-ras oncogene transformed tumors by inhibiting the membrane association of tumoral p21(H-ras) and that the allyl group is an important determinant in tumor growth inhibitory effect of DADS.

Thomson M, Ali M. 2003. Curr Cancer Drug Targets. 3(1):67-81.

Numerous studies have demonstrated the chemopreventive activity of garlic by using different garlic preparations, including fresh garlic extract, AGE, garlic oil and a number of organosulfur compounds derived from garlic. Recent research has also focused on the antimutagenic activity of garlic. It has also been observed that AGE, but not fresh garlic extract, exhibits radical scavenging activity. The two major compounds in AGE, *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC) have the highest radical scavenging ability. Because of this, consumption of garlic may provide protection from cancer development.

Inhibited the Growth of Bladder Tumors

Lau BHS, Barker GR, et al. 1985. 85th Annual Meeting of the American Society for Microbiology. Las Vegas, NV. Mar 3-7. Abst #E-95.

Marsh CL, Torrey RR, et al. 1987. J Urol. 137(2):359-62.

Lau and Marsh et al. observed that AGE was equally or slightly more effective than *Bacillus Calmette-Guerin* (BCG) at inhibiting the development and growth of intravesically transplanted bladder tumors. AGE given 1 and 6 days after transplantation of bladder tumor cells yielded the lowest final tumor incidence.

Lau BHS, Woolley J, et al. 1986. J Urol. 136(3):701-5.

AGE was found to be more effective than *Bacillus Calmette-Guerin* (BCG) at inhibiting the development and growth of bladder tumors from implanted transitional cell carcinoma.

Lamm DL, Rigg DR, et al. 1990. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. Aug 28-30, p. 30.

At dosages of 6.3, 12.5 and 25.0 µg, AGE significantly decreased murine bladder carcinoma cells (MBT2) incidence by 28.6%, 44.4% and 28.6%, respectively. AGE was as effective as *Bacillus Calmette-Guerin* (BCG). Tumor volume was also significantly decreased by AGE. Lamm et al. concluded that AGE will prove to be a highly effective form of immunotherapy for the treatment of transitional cell carcinoma of the bladder.

Riggs DR, Lamm DL, et al. 1995. J Urol. 153(4):Abst #1029

AGE was found to be a "highly impressive nontoxic oral treatment modality" in a bladder cancer model. The effect was similar to that of *Bacillus Calmette-Guerin* (BCG). At a low dosage of 50 mg of AGE, it significantly reduced tumor volume, but not mortality. At a higher dosage of 500 mg, it significantly reduced both tumor volume and mortality. No adverse effects were noted in any AGE groups.

Lamm DL, Riggs DR, et al. 2001. J Nutr. 131(3 Suppl):1067S-70S.

AGE administered intralesionally and orally to models that received subcutaneous implantation of murine bladder carcinoma cells (MBT2). Both intralesional and oral AGE significantly reduced tumor volume and mortality. The anti-tumor efficacy of garlic has been attributed to inhibition of chemical carcinogenesis and direct cytotoxic and cytostatic effects, but some evidence suggests that garlic has an immunopotentiating effect as well. Immune effects of garlic reported in literature include activation of macrophages and enhancement of cytotoxicity and phagocytosis, stimulation of lymphocyte proliferation, and increased infiltration of macrophages and lymphocytes into tumor cells. Diallyl disulfide (DADS) inhibits leukocytes count suppression caused by chemotherapy. In patients with AIDS, garlic treatment is reported to increase natural killer (NK) cell activity. A protein fraction (F-4) is reported to enhance the ability of peripheral blood lymphocytes to destroy a variety of tumor cell lines. Garlic is a complex agent and the observed anti-tumor activity is likely the result of multiple mechanisms.

Hu H, Zhang XP, et al. 2011. Mol Med Report. 4(1):9-16.

Studies have shown that expression of inhibitor of differentiation (Id-1) is increased in bladder cancer and is associated with drug resistance. Results of this study show that overexpression of Id-1 was shown to reduce the positive effect of *S*-allylmercaptocysteine (SAMC) on cell survival, while inactivation of Id-1 increased cellular susceptibility to SAMC. Other tests confirmed the apoptosis of bladder cancer cells induced by SAMC was shown to be negatively regulated by Id-1 expression. The inhibitory effect of SAMC on the invasion and migration of bladder cancer cells was found to be associated with the down-regulation of Id-1. Results demonstrated that SAMC-induced apoptosis is associated with the Id-1 pathway, and that the inactivation of Id-1 enhances the ability of SAMC to inhibit the survival, invasion

and migration of bladder cancer cells.

Inhibited the Growth of Melanoma Cells

Hoon DSB, Sze L, et al. 1990. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. Aug 28-30, p. 26.

According to Hoon et al., melanoma, a skin cancer, has one of the fastest increasing incidence rates of all cancer in humans. It divides and spreads rapidly and is difficult to treat when malignant. In the study, AGE significantly inhibited (>50%) the *in vitro* growth of melanoma cell lines. Furthermore, equivalent concentrations of AGE did not inhibit the growth of beneficial lymphocytes.

Takeyama H, Hoon DSB, et al. 1993. Oncology. 50(1):63-9.

S-allyl cysteine (SAC) inhibited the proliferation of nine human melanoma cell lines and one murine melanoma cell line in a dose-dependent manner. SAC inhibited cellular growth and proliferation and modulated major cell differentiation marker of melanoma.

Inhibited the Growth of Neuroblastoma Cells

Welch C, Wuarin L, et al. 1992. Cancer Lett. 63(3):211-9.

S-allyl cysteine (SAC) inhibited the growth of neuroblastoma (nerve) cells. The effects were observed in a time- and dose-dependent manner, when SAC was cultured in these cells for 2 days.

Induce Apoptosis of Neuroblastoma Cancer Cells

Kanamori Y, Via LD, et al. 2020. Exp Ther Med. 19(2):1511-21.

This study examined the effects of AGE on mitochondria isolated from liver mitochondria from a model and AGE and *S*-allyl-L-cysteine (SAC) on human neuroblstoma (NB) cancer cells. Results indicate that both natural products, AGE and SAC, cause cytotoxicity to tumor cells via the induction of mitochondrial permeability transition (MPT).

Inhibited Tumor Activity Against Primary Effusion Lymphoma (PEL)

Shigemi Z, Furakawa Y, et al. 2016. Int J Oncol. 48(1):293-304.

Diallyl trisulfide (DAT) inhibited nuclear factor-κB (NF-κB) signaling and induced apoptosis through destabilization of tumor necrosis factor receptor associated factor 6 (TRAF6) in primary effusion lymphoma (PEL), a subtype of non-Hodgkins's B-cell lymphoma, *in vitro* and *in vivo*.

Inhibited the Growth of Sarcoma Cells

Hu X, Cao G, et al. 2002. Int J Mol Med. 9(6):641-3.

The study indicated that sarcoma cell growth after 5 days was 20 x 10^4 in the control group compared to 7.3 x 10^4 and 14.3 x 10^4 in groups treated with 20 mg/ml and 10 mg/ml of AGE, respectively. A reduction in cell migration was also seen between the control and AGE (20 mg/ml) groups, from 7.44 mm to 2.48 mm. This is the first report to demonstrate AGE's inhibitory effects of growth and migration of sarcoma cells. Reducing the movement of sarcoma cells would inhibit metastasis, thereby making surgical removal of tumors more effective.

Suppressed Proliferation of Ovarian Cancer Cells

Xu YS, Feng JG, et al. 2014. Acta Pharmacol Sin. 35(2):267-74.

S-allyl cysteine (SAC) (1-100 mmol/L) was shown to inhibit the proliferation of human epithelial ovarian cancer cell line A2780 cells *in vitro* in dose- and time-dependent manners (the IC_{50} value was approximately 25 mmol/L at 48 hours, and less than 6.25 mmol/L at 96 hours). Furthermore, SAC dose-dependently inhibited the colony formation of A2780 cells. SAC treatment resulted in G1/S phase arrest and induced apoptosis, accompanied by decreased expression of procaspase-3, Parp-1 and Bcl-2, and increased expression of active caspase-3 and Bax. SAC treatment also significantly reduced the migration of A2780 cells, and markedly decreased protein expression of Wnt5a, p-AKT and c-Jun, which were the key proteins involved in proliferation and metastasis.

Wu J, Zhao S, et al. 2016. Exp Mol Pathol. 100(2):294-302.

In vivo and *in vitro* experiments using three ovarian cancer cell lines were subjected to *S*-allylmercaptocysteine (SAMC) treatment. SAMC suppresses both the proliferation and distant metastasis of epithelial ovarian cancer cells, and insensitivity to one of the cell lines to SAMC was closely related to the high level of survivin expression and that combination of SAMC treatment together with survivin knockdown might be a potential strategy for treating certain variants of ovarian cancers.

Inhibited the Development of Carcinogen-Induced Skin Cancer

Nishino H, Iwashima A, et al. 1989. Oncology. 46(4):277-80.

Nishino H, Nishino A, et al. 1990. Cancer J. 3(1):20-1.

AGE was found to inhibit the development of skin cancer induced by the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA). It was also observed to inhibit the promoter 12-O-tetradecanoyl (TPA). *In vitro*, AGE inhibited tumor promoter 12-O-tetradecanoylphorbol 13-acetate (TPA), which is responsible for the earliest stages of cancer development by enhancing phospholipid metabolism. *In vivo*, AGE suppressed the first stage of tumor promotion in two-stage skin carcinogenesis. AGE appeared to be effective in inhibiting the initial events caused by TPA type tumor promoters both *in vitro* and *in vivo*. Allixin, one of the compounds in AGE, demonstrated similar chemopreventive effects following exposure to DMBA and TPA.

Reeve VE, Bosnic M, et al. 1993. Photochem Photobiol. 58(6):813-7.

AGE protected bald models from photocarcinogenesis or skin carcinogenesis induced by ultraviolet B (UVB) radiation. AGE-treated models exposed to the cancer-causing agent 7,12-dimethylbenz(a)anthracene (DMBA) then exposed to UVB radiation for 6 weeks also developed significantly fewer tumors than untreated models exposed to the same regimen.

Inhibited the Growth of Carcinogen-Induced Tumors of the Breast

Milner JA, Liu JZ. 1990. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. Aug 28-30, p. 25.

In a 7,12-dimethylbenz(a)anthracene (DMBA) treatment while receiving a diet of 4% AGE, mammary cancer incidence was 35% compared to 90% in controls receiving no AGE. The 4% diet was given 2 weeks prior to and 2 weeks following DMBA treatment. AGE also significantly inhibited *in vivo* formation of adducts, which were 96% correlated to ultimate tumor number. This research suggests that AGE could alter the mammary tissue's ability to convert DMBA into an active cancer-causing agent, which then can bind to DNA.

Liu JZ, Milner J. 1990. FASEB J. 4:Abst #5227.

Treatment with AGE (4% of diet) and selenium (1 mcg/g) resulted in synergistic 30% reduction in DNA adduct formation exposed to the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA).

Liu JZ, Lin RI, et al. 1992. Carcinogenesis. 13(10):1847-51.

Liu JZ, Lin XY, et al. 1992. FASEB J. 6(4):Abst #3230.

AGE was found to significantly delay the onset of the first tumors and reduced the final mammary tumor incidence in models exposed to the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA). AGE also reduced DMBA-DNA adduct formation by reducing DMBA-DNA binding. Glutathione *S*-transferase (GST) activity increased in both the liver and mammary tissues of models taking AGE.

Zoumas C, Amagase H, et al. 1992. FASEB J. 6(4):Abst #2641.

A diet supplemented with 2% AGE depressed 7,12-dimethylbenz(a)anthracene (DMBA)-DNA binding by 30-40%. AGE given in accordance with a high-fat diet (20% of total calories) depressed binding by 56%. An increase in DMBA-DNA binding is associated with an increase in fat content of the diet.

Amagase H, Milner J. 1992. FASEB J. 6(4):Abst #3229.

Amagase H, Milner J. 1993. Carcinogenesis. 14(8):1627-31.

The majority of the garlic preparations tested could reduce the binding of the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) to mammary cell DNA. However, among the effective garlic preparations, only AGE did not cause any side effects. The other garlic preparations, including commercial garlic powder, significantly decreased food intake and body weight gain. *S*-allyl cysteine (SAC) also dose-dependently reduced DMBA-DNA adduct formation in the mammary gland.

Sundaram SG, Milner JS. 1992. FASEB J. 6(4):Abst #2639.

Sundaram SG, Milner JS. 1993. Cancer Lett. 74(1-2):85-90.

The oil-soluble sulfur compounds diallyl sulfide (DAS) and diallyl disulfide (DADS) inhibited mammary tumor cell growth by 30% and 37%, respectively. Diallyl trisulfide (DATS) inhibited tumor growth by 50% and increased dosages inhibited tumor growth by 81%.

Tiwari RK, Pinto J, et al. 1993. Breast Cancer Res Treat. 27(1/2):Abst #80.

Li G, Qiao CH, et al. 1995. Oncol Rep. 2(5):787-91.

S-allyl mercaptocysteine (SAMC) inhibited the growth and proliferation of transformed human breast cells. They also increased both glutathione *S*-transferase and peroxidase levels in the non-transformed cells.

Lin XY, Liu JZ, et al. 1994. Carcinogenesis. 15(2):349-52.

Sodium nitrite and aminopyrine given to models results in the generation of carcinogenic *N*-nitroso-compounds (NOC). Simultaneous supplementation with AGE at 2% and 4% of their diets reduced the occurrence of NOC-induced liver DNA adducts by 40% and 60%, respectively. The mammary tissue DNA adducts were reduced by 50% and 70%, respectively. AGE may have depressed the formation of NOC from these precursors and also decreased the toxicity of NOC, thus inhibiting its ability to generate adducts.

Liu JZ, Schaffer EM, et al. 1995. FASEB J. Abst #95.

AGE and two of its constituents *S*-allyl cysteine (SAC) and diallyl disulfide (DADS) are effective inhibitors of *N*-methylnitrourea-induced mammary tumors. Final tumor incidence was 81% for the control group, 19% for the AGE group, 38% for the SAC group and 38% for the DADS group.

Schaffer EM, Liu JZ, et al. 1996. Experimental Biology. Abst #96.

Schaffer EM, Liu J, et al. 1997. Nutr Cancer. 27(2):162-8.

AGE, *S*-allyl cysteine (SAC) and diallyl disulfide (DADS) inhibited the initiation of 7,12-dimethylbenz(a)anthracene (DMBA) mammary carcinogenesis by 40%, 80% and 75%, respectively. Selenium also appeared to enhance the activity of each of these compounds suggesting synergism.

Amagase H, Schaffer EM, et al. 1996. J Nutr. 126(4):817-24.

AGE was found to reduce the development of DNA adducts induced by the mammary or breast carcinogen 7,12-dimethylbenz(a)anthracene (DMBA), regardless of the protein content of the diet. Increasing fat with corn oil in diets increased DMBA-DNA adduct formation, but when AGE was added to the diet, it inhibited the rise in adduct formation caused by the corn oil. Diets supplemented with selenium, vitamin A and AGE were less prone to DNA adduct formation than those supplemented with only one of the above ingredients. This outcome suggests a synergistic effect among the three compounds.

Schaffer EM, Liu J, et al. 1997. Cancer Lett. 102(1-2):199-204.

S-allyl cysteine (SAC) and diallyl disulfide (DADS) are effective inhibitors of *N*-methyl-*N*-nitrosourea (MNU)-induced mammary carcinogenesis. Garlic powder, SAC and DADS supplementation significantly delayed the onset of mammary tumors compared to the control group. Tumor incidence 23 weeks after MNU treatment was reduced by 76%, 41% and 53% in models fed garlic, SAC and DADS, respectively. Also the quantity of mammary DNA alkylation occurring 3 hours after MNU treatment was reduced, specifically, O^6-methylguanine adducts were reduced by 27%, 18% and 23% for garlic powder, SAC and DADS respectively, and N^7-methylguanine 23% adducts decreased by 48%, 22% and 21%, respectively.

Schaffer EM, Milner JA. 1997. 16th International Congress of Nutrition. IUNS. Jul 27-Aug 1, p. 67. Abst #PW 10.13.

Models fed additional linoleic acid or oleic acid (to a standard 5% corn oil diet) had approximately 60% and 35% more ($P<0.05$) total mammary DNA adducts, respectively, than those fed the basal diet but only 10% and 20% more adducts, respectively, when 2% AGE was also supplemented in the diet. This research concluded that AGE could block the enhancement by specific fatty acids of the initiation of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary carcinogenesis.

Song K, Milner J. 1999. J Nutr. 129(3):657-61.

By providing either 0.105 µmol if diallyl disulfide (DAS) or *S*-allyl cysteine (SAC) by gastric gavage thrice weekly for 2 weeks was effective in retarding 7,12-dimethylbenz(a)anthracene (DMBA) bioactivation.

Pinto J, Rivlin R. 2001. J Nutr. 131(3 Suppl):1058S-60S.

Garlic constituents have been shown to inhibit both initiation and promotion of cancer and have attracted wide interest in their anti-cancer potential. Studies were conducted with human breast cancer cell lines estrogen-sensitive (MCF-7) and estrogen-insensitive (MCF-ras) and found that AGE, *S*-allyl cysteine (SAC) and *S*-allyl mercaptocysteine (SAMC) inhibit cell growth progressively at increasing concentrations.

Gapter LA, Yuin OZ, et al. 2008. Biochem Biophys Res Commun. 367(2):446-51.

S-allyl cysteine (SAC) significantly reduced anchorage-dependent and –independent growth of breast tumor cells in a dose- and time-dependent fashion and sub-lethal SAC treatment altered mammary tumor cell adhesion and invasion through components of the extracellular matrix. Evidence provided suggests increased expression of E-cadherin and reduced matrix metalloproteinase-2 (MMP-2) expression and activity are partially responsible for inhibition of mammary tumor cell invasion by SAC.

Anti-Cancer Activity in Breast Cancer Biomarkers

Roy N, Davis S, et al. 2016. Asian Pac J Cancer Prev. 17(6):2883-8.

Twelve compounds in garlic, including S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC) were found to be active against breast cancer (BC) target proteins or biomarkers.

Inhibited the Growth of Carcinogen-Induced Tumors of the Colon

Wargovich MJ. 1987. Carcinogenesis. 8(3):487-9.

Diallyl sulfide (DAS), a constituent in AGE, significantly reduced the incidence of colon cancer by 74%, induced by 20 weekly injections of the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA).

Sumiyoshi H, Wargovich MJ. 1989. Proc Am Assoc Cancer Res. 30:181. Abst #718.

Sumiyoshi H, Wargovich MJ. 1990. Cancer Res. 50(16):5084-7.

Two constituents in AGE, diallyl disulfide (DADS) or *S*-allyl cysteine (SAC), significantly inhibited nuclear damage caused by the carcinogen dimethylhydrazine (DMH), thus decreasing the toxicity of this carcinogen. Both compounds also significantly stimulated the activity of glutathione *S*-transferase (GST) in both the liver and colon.

Sundaram SG, Milner JS. 1996. J Nutr. 126(5):1355-61.

Diallyl disulfide (DADS) was found to be more effective than 5-flurorouracil (5-FU), a common anti-cancer drug, at inhibiting the growth of human colon tumor cells, especially when injected intraperitoneally. DADS given simultaneously with 5-FU prevented some of the side effects induced by 5-FU such as depression of white blood cells, spleen weight and elevated plasma urea.

Hatono S, Jimenez A, et al. 1996. Carcinogenesis. 17(5):1041-4.

Aberrant crypt foci (ACF) are considered to be the most likely precursors of colon cancer. *S*-allyl cysteine (SAC) administration inhibited the development in the colon of 1/3 to ½ of the ACF induced by dimethylhydrazine (DMH) when given prior to this carcinogen (initiation phase). SAC was found to significantly enhance glutathione *S*-transferase (GST) activity not only in the liver but also in the proximal and middle small bowel. Thus, SAC inhibited the development of pre-cancerous lesions in the colon and enhanced the activity of enzyme systems in the liver and small intestine, which detoxify carcinogens.

Uda N, Wargovich M, et al. 1996. Proc Am Assoc Cancer Res. 37:Abst #1871.

Additional research found that AGE could change the metabolism of the carcinogen azoxymethane (AOM) and this inhibited AOM-induced aberrant crypt foci (ACF). Models were given AOM 15 mg/kg/week, i.p., for 2 weeks. In the initiation study, they were also given AGE 120, 600 or 3000 mg/kg for 3 consecutive days one week prior to and including the 2 weeks exposure to AOM. AGE significantly inhibited AOM-induced ACF. When AGE was taken 2 weeks after AOM treatment for 4 weeks (post-initiation), it had only a mildly preventive effect.

Hatono S, Wargovich MJ. 1997. Ch. 15. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 139-51.

The administration of 0.4 and 0.8 maximum tolerated dose of *S*-allyl cysteine (SAC) incorporated into the experimental diet significantly decreased the number of aberrant crypt foci (ACF) when given during the initiation but not promotion induced by the carcinogen dimethylhydrazine (DMH) or azoxymethane (AOM). Models given *S*-ethylmercaptocysteine, *S*-propylmercaptocysteine and *S*-propaglylcysteine, exhibited increased ACF, which is determined to be due to decreased food intake caused by these compounds.

Knowles LM, Milner JA. 1997. FASEB J. 11(3):A422. Abst #2445.

Diallyl disulfide (DADS) and *S*-allyl mercaptocysteine (SAMC) are effective at suppressing growth of cultured human colon tumor cells. At equimolar amounts, DADS was most effective. DADS (25 µg) and SAC (300 µg) caused a 23% suppression in cell growth. DADS also assisted cells in converting from a mutated to a normal state (from G1 to S phase).

Shirin H, Pinto JT, et al. 2001. Cancer Res. 61(2):725-31.

S-allyl mercaptocysteine (SAMC), but not *S*-allyl cysteine (SAC), inhibited the growth of two human colon cancer cell lines SW-480 and HT-29 at doses similar to that of sulindac sulfide (SS). SAMC also induced apoptosis. The effects of SAMC were accompanied by a marked increase in endogenous levels of reduced glutathione. SAMC when co-administered with SS, enhanced the growth inhibitory and apoptotic effects of SS. These findings suggest that SAMC may be useful in colon cancer prevention when used alone or in combination with SS or other chemopreventive agents.

Xiao D, Pinto JT, et al. 2003. Cancer Res. 63(20):6825-37.

Experimental carcinogenesis studies indicate that components of garlic (i.e., allyl sulfides) inhibit both the initiation and promotion stages of tumorigenesis for various types of cancer, including colorectal cancer. It was previously reported that *S*-allyl mercaptocysteine (SAMC) inhibits growth, arrests cells in Gap 2 phase/mitosis phase (G_2/M) and induces apoptosis in human colon cancer cells. This study concludes that the garlic-derived compound SAMC exerts antiproliferative effects by binding directly to tubulin and disrupting the microtubule (MT) assembly, thus arresting cells in mitosis and triggering c-Jun NH_2-terminal kinase 1 (JNK1) and caspase-3 signaling pathways that lead to apoptosis.

Ross SA, Finley JW, et al. 2006. J Nutr. 136(3 Suppl):852S-4S.

Models were provided a semi-purified diet, casein-based diet with or without 57 or 570 µmol/kg of *S*-allyl cysteine (SAC), diallyl disulfide (DADS) or *S*-allyl mercaptocysteine (SAMC) for 13 weeks prior to determination of aberrant crypt foci (ACF) and aberrant crypt number. All treatments except 57 µmol/kg, significantly lowered ACF compared to controls. ACF was significantly reduced by DADS and SAMC at both concentrations tested. This study revealed all allyl sulfur compounds are not equivalent in retarding early preneoplastic markers for colon cancer.

Katsuki T, Hirata K, et al. 2006. J Nutr. 136(3 Suppl):847S-51S.

Models were given weekly subcutaneous injections of dimethylhydrazine (DMH) for 20 weeks and were fed with either a normal or AGE-containing diet. AGE significantly reduced in number of amount of colon tumors and aberrant crypt foci (ACF) compared to the normal diet. AGE treatment was shown to cause a significant decrease in cell proliferation of normal appearing colonic mucosa suggesting that AGE has antiproliferative action on colorectal carcinoma cells and an inhibitory action on angiogenesis.

Bao JO, Yuk DY, et al. 2007. J Pharmacol Sci. 104(4):374-83.

Thiacremonone, a sulfurcompound isolated from garlic, was shown to inhibit colon cancer cell (SW620 and HCT116) growth followed by induction of apoptosis in a dose-dependent manner. Thiacremonone

also modulated tumor necrosis factor-α (TNF-α) and tetradeanoyl phorbol acetate (TPA)-induced nuclear factor κB (NFκB) transcriptional and DNA binding activity. Moreover, thiacremonone suppressed NFκB target anti-apoptotic genes (Bcl-2 [B cell lymphoma 2], cIAP1/2 [baculoviral inhibitor of apoptosis repeat-containing protein ½] and XIAP [X-linked inhibitor of apoptosis protein]) and inflammatory genes (iNOS [inducible nitric oxide synthase] and COX-2 [cyclooxygenase-2]), whereas it induced apoptotic genes (Bax [B-cell lymphoma-2-associated X protein], cleaved caspase-3 and cleaved PARP [poly(ADP-ribose) polymerase]) expression. These results suggest that a novel sulfurocompound from garlic inhibited colon cancer cell growth through induction of apoptotic cell death by modulating of NFκB.

Liang D, Qin Y, et al. 2011. Cancer Lett. 310(1):69-76.

S-allylmercaptocysteine (SAMC) could effectively suppress the growth and metastasis of colorectal cancer (CRC) cells both *in vivo* and *in vitro*. The anticancer effect of SAMC was related to the decreased proliferation and increased apoptosis as well as necrosis of cancer cells. Taken together, the proliferation and metastasis of CRC cells can be significantly suppressed by SAMC treatment under both *in vitro* and *in vivo* conditions, thus, SAMC may be a promising candidate for CRC chemotherapy.

Jikhara H, Qi G, et al. 2015. Oncol Rep. 33(3):1131-40.

Models with carcinogenesis were induced by 1,2-dimethylhydrazine (DMH) for 8 weeks. Those that moved to a basal diet containing 3% wt/wt AGE were shown to decrease the number of aberrant crypt foci (ACF) and lower number of adenoma and adenocarcinoma lesions. Data indicated that AGE suppressed the proliferative activity in adenoma and adenocarcinoma lesions and that AGE delayed cell cycle progression by downregulating cyclin B1 and cdk1 expression via inactivation of nuclear factor-kappaB (NF-κB) in the human colorectal cancer cells.

Inhibited the Growth of Carcinogen-Induced Tumors of the Esophagus

Wargovich MJ, Woods C, et al. 1988. Cancer Res. 48(23):6872-5.

Diallyl sulfide (DAS) totally suppressed the development of carcinogen *N*-nitrosomethylbenzylamine (NMBA)-induced esophageal tumors (P>0.0001).

Inhibited the Growth of Carcinogen-Induced Tumors/Cancer of the Stomach and Lung

Sparnins VL, Motts AW, et al. 1986. Nutr Cancer. 8(3):211-5.

Allyl methyl trisulfide (AMT), an organosulfur compound in AGE, could increase glutathione *S*-transferase (GST) activity in the forestomach, small bowel mucosa, liver and lung of models when given orally. AMT also inhibited benzo-(a)-pyrene (BP)-induced neoplasia of the forestomach as shown by a greater than 70% reduction in the number of tumors.

Sparnins VL, Barany G, et al. 1988. Carcinogenesis. 9(1):131-4.

Several organosulfur compounds in AGE, allyl methyl trisulfide (AMT), allyl methyl disulfide (AMD), diallyl trisulfide (DAT) and diallyl sulfide (DAS), inhibited benzo-(a)-pyrene (BP)-induced neoplasia of the forestomach. DAS was more effective than AMT. DAS and AMD also inhibited pulmonary adenoma formation (lung cancer). From the study, it appeared that the number of sulfur atoms in the organosulfur compound could determine the organosulfur compounds sites at which protection against carcinogenesis would occur. All four compounds induced glutathione *S*-transferase (GST) activity in the forestomach but varied in their capacity to induce GST activity in the lung, liver and small bowel.

Wattenberg LW, Sparnins VL, et al. 1989. Cancer Res. 49(10):2689-92.

Diallyl disulfide (DADS) and allyl methyl disulfide produced a marked inhibition of *N*-nitrosodiethylamine (NDEA)-induced tumors of the forestomach when given 96 and 48 hours prior to NDEA. DADS reduced tumor formation by more than 90%. Pulmonary adenoma formation was inhibited by about 30%. When given 15 minutes to one hour prior to NDEA, DADS and allyl mercaptan, a metabolic product of DADS, inhibited forestomach tumor formation by greater than 75% and lung cancer formation by more than 20%.

Sakamoto K, Lawson LD, et al. 1996. FASEB Meeting. Washington, D.C. Abst #2868.

Diallyl trisulfide (DATS) is extremely effective in reducing the growth of human lung carcinoma cells in cultures. Treatment with 10 and 50 µM DATS for 24 hours reduced the growth by 47% and 72%, respectively, making it 10 times more effective than DADS.

El-Bayoumy K, Raghu S, et al. 2006. J Nutr. 136(3 Suppl):864S-9S.

Selenium compounds in garlic exhibited chemopreventive effects by inhibiting the development of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary adenocarcinomas and azoxymethane-induced colon cancer and benzo[a]pyrene-induced forestomach tumors.

Wang K, Wang Y, et al. 2016. Int Immunopharmacol. 34:37-43.

S-allylmercaptocysteine (SAMC) was shown to inhibit benzo(a)pyrene (B(a)P) carcinogenesis in human

lung cells (A549 cell line) through mechanisms that include suppression of cell proliferation, cell cycle regulation, attenuation of reactive oxygen species (ROS) formation, inhibition of DNA damage, increase of superoxide dismutase (SOD) activity and inhibition of nuclear factor-kappa B (NF-κB) activity.

Inhibited the Growth of Prostate Cancer Cells

Pinto J, Qiao C, et al. 1997. FASEB J. 11(3):A439. Abst #2541.

S-allyl mercaptocysteine (SAMC) may inhibit the growth of certain prostate cancer cells. The cells used in their study were androgen-responsive prostate cancer cells (LNCaP). Testosterone, specific androgen or male hormone, enhances the activity/growth of LNCaP. SAMC was found to enhance the catabolism or degradation of testosterone. Therefore, it was suggested that SAMC, by catabolizing testosterone, hampered the progression or activation of prostate cancer cells. Prostate specific antigen (PSA) levels were also markedly reduced after treatment with SAMC. PSA is considered a marker for prostate cancer.

Pinto JT, Qiao C, et al. 1997. Am J Clin Nutr. 66(2):398-405.

S-allyl mercaptocysteine (SAMC), a constituent found only in AGE, inhibits the growth of human prostate cancer cells (LNCaP). *S*-allyl cysteine (SAC) also had some effects but was not as pronounced. Both SAMC and SAC increased reduced glutathione in LNCaP. Reduced glutathione may hinder cell growth by impeding enzymes such as ornithine decarboxylase, necessary for synthesis. SAMC may also inhibit this enzyme directly.

Sigounas G, Hooker J, et al. 1997. Nutr Cancer. 27(2):186-91.

S-allyl mercaptocysteine (SAMC) in AGE could inhibit the growth of the hormone responsive prostate cancer cell line (CRL-1740). Even at 0.05 mM, SAMC totally suppressed the growth of CRL-1740 cells compared with the solvent-treated control cells.

Pinto JT, Qiao CH, et al. 2000. The Prostate. 45(4):304-14.

S-allyl mercaptocysteine (SAMC) affected biomarkers of human prostate cancer cells (LNCaP) in a way similar to androgen or hormone deprivation. It was suggested that SAMC diminishes the trophic or cancer growth-enhancing effects of testosterone, likely by converting it to metabolites less reactive toward androgen receptors that are less likely to enhance the growth of cancer. Specifically, SAMC significantly reduced prostate specific antigen (PSA) secretions from LNCaP after 1, 4 and 6 days of exposure and increased prostate specific membrane antigen (PSMA) activity. SAMC added to LNCaP enhanced the rate of testosterone disappearance from the culture media at 6 hours ($p<0.01$).

Pinto J, Rivlin R. 2001. J Nutr. 131 (3 Suppl):1058S-60S.

S-allyl mercaptocysteine (SAMC) was shown to markedly inhibit the rate of growth of human prostate cancer cells (LNCaP) at 4, 6 and 8 days of incubation. When SAMC is removed from the medium and replaced with phosphate buffered saline, persistent inhibitory effects upon rate of cell growth occur. Inhibition of growth of LNCaP is also achieved with *S*-allyl cysteine (SAC) and a variety of lipid-soluble derivatives. SAMC reduces secretion into the media of prostate specific antigen (PSA) and elevates activity of a new biomarker, prostate specific membrane antigen (PSMA), findings compatible with an androgen deficient state.

Wang T, Milner M, et al. 2003. FASEB J. 17(4):A376. Abst #270.1.

Human prostate cancer cells (LNCaP) was utilized as an *in vitro* model and treated the cells with diallyl disulfide (DADS). The complementary DNA (cDNA) microarray approach to profile gene expression changes upon treatment with the garlic-derived cancer preventive compound DADS were explored.

Chu Q, Ling MT, et al. 2006. Carcinogenesis. 27(11):2180-9.

Metastatic cancer is one of the main causes of cancer-related deaths since it rarely responds to available treatments. Using colony-forming, wound-closure as well as matrigel-invasion assays, it was found that the two main water-soluble constituents of garlic *S*-allyl cysteine (SAC) and *S*-allylmercaptocysteine (SAMC), were able to suppress potentially invasive androgen-independent prostate cancer (Pca) cell proliferation and invasive abilities through restoration of E-cadherin expression in cancer cells.

Howard EW, Ling MT, et al. 2007. Clin Cancer Res. 13(6):1847-56.

S-allylmercaptocysteine (SAMC) was shown not only to inhibit the growth of primary tumors by up to 71% ($P<0.001$) in androgen-independent prostate cancer model, but also reduced the number of lung and adrenal metases by as much as 85.5% ($P=0.001$) without causing notable toxicity. The metastatic suppression was accompanied by a 91% reduction of viable circulating tumor cells ($P=0.041$), suggesting that SAMC prevents the dissemination by decreasing tumor cell intravasation.

Liu Z, Li M, et al. 2012. Mol Med Report. 5(2):439-43.

Androgen-independent human prostate cancer (PC-3) cells were incubated with *S*-allyl cysteine (SAC) at three different concentrations. SAC suppressed the proliferation of PC-3 cells and led to cell cycle arrest at the Gap 0/Gap 1 (G0/G1) phases, as well as inducing cell apoptosis which was accompanied by the decreased expression of B-cell lymphocyte 2 (Bcl-2) and increased expression of Bcl-2 associated X protein (Bax) and caspase 8.

Inhibited Lung Cancer Proliferation

Tang FY, Chiang EP, et al. 2010. J Agric Food Chem. 58:11156-64.

It was found that *S*-allyl cysteine (SAC) significantly inhibited the proliferation of human non-small-cell lung carcinoma (NSCLC) A-549 cells and significantly suppressed the activation of mammalian target of rapamycin (mTOR), nuclear factor κB (NF-κB) and cyclin D1 molecules *in vitro*. Furthermore, SAC significantly inhibited the growth of highly metastatic human NSCLC cells in tumor-bearing models, indicating that SAC could effectively suppress the growth and malignant progression of human NSCLC and were associated with suppression of mTOR and NF-κB molecules *in vivo*.

Inhibited Oral Cancer Progression

Tang FY, Chiang EP, et al. 2009. J Nutr Biochem. 20(12):1013-20.

S-allyl cysteine (SAC) dose-dependently inhibited the growth of human oral squamous cancer (CAL-27) cells and induced the expression of E-cadherin adhesion molecule. Other results revealed that SAC could restore the distribution of E-cadherin molecule on cell membrane, stabilized the adherent junction complex of E-cadherin/beta-catenin in oral cancer cells and significantly inhibited the activation of mitogen-activated protein kinases/extracellular signal-regulated kinases (MAPK/ERK) signaling pathway. These findings were associated with the down-regulation of the zinc finger protein (SLUG) or SNAI2 repressor protein.

Pai MH, Kyo YH, et al. 2012. Br J Nutr. 2011. 108(1):28-38.

S-allyl cysteine (SAC) dose-dependently inhibited the growth of oral cancer in tumor-bearing models. The histopathological and immunohistochemical staining results indicated that SAC was able to effectively suppress tumor growth and progression of oral cancer *in vivo*.

Inhibited Growth of Erythroleukemia Cell Lines

Sigounas G, Hooker J, et al. 1997. Nutr Cancer. 27(2):186-91.

S-allyl mercaptocysteine (SAMC) was found to inhibit the growth of two human erythroleukemia cell lines HEL and OCIM-1. HEL cells showed complete suppression of growth at ≥ 0.025 mM SAMC and even 0.1 mM SAMC inhibited HEL cell growth by more than 70%. OCIM-1 cells exhibited a 55% reduction in growth at 0.1 mM SAMC.

Sigounas G, Hooker J, et al. 1997. Nutr Cancer. 28(2):153-9.

S-allyl mercaptocysteine (SAMC) was further confirmed to inhibit the growth of two erythroleukemia cell lines HEL and OCIM-1. It induced a dose-dependent inhibition with a 50% lethal dose of 0.046 mM for CIM-1 cells and 0.093 mM for HEL cells. The authors concluded from analyses of [^{3}H] thymidine incorporation and high molecular weight DNA fragmentation that SAMC is an effective antiproliferative agent against erythroleukemia cells that induces death by apoptosis.

Inhibited Aflatoxin B_1 and Benzo[a]pyrene-Induced Mutagenesis

Tadi PP, Teel RW, et al. 1990. Int Clin Nutr Rev. 10:423-9.

AGE and its constituents were found to inhibit aflatoxin B_1 (AFB_1) from binding to DNA, preventing the formation of AFB_1-DNA adducts. AGE prevented AFB_1 from being converted into active cancer-causing compounds in the body by enhancing its conversion to non-toxic conjugates. AGE also prevented benzo[a]pyrene-induced mutagenesis. Benzo[a]pyrene is found in cigarette smoke, charcoal broiled meat and automobile exhaust and is a procarcinogen causing a number of model tumors.

Tadi PP, Teel RW, et al. 1991. Nutr Cancer. 15(2):87-95.

AGE and one of its constituents, diallyl sulfide (DAS), inhibited mutagenesis induced by aflatoxin B_1 (AFB_1). These compounds inhibited AFB_1 binding to DNA. AGE also significantly decreased noxious metabolites of AFB_1 and increased glucuronide and glutathione (detoxified metabolites of AFB_1). Tadi et al. concluded that AGE was antimutagenic and potentially anticarcinogenic.

Yamasaki T, Teel RW, et al. 1991. Cancer Lett. 59(2):89-94.

Allixin, a constituent in AGE, inhibited the binding of the carcinogenic aflatoxin B_1 (AFB_1) to thymus DNA and reduced the formation of AFB_1-DNA adducts. Allixin inhibited the formation of carcinogenic metabolites of AFB_1 and it was suggested that this compound may be useful in the chemoprevention of cancer.

Suppressed Activity of Procarcinogen-Activating Enzymes

Dion ME, Milner JA. 1997. FASEB J. 11(3):A370. Abst #2144.

The ability of AGE and one of its constituents to inhibit the enzyme system in the cell responsible for generating toxic metabolites from procarcinogens (cytochrome P450 2E1 [CYP 2E1]) is determined. Models were given AGE and diallyl disulfide (DADS) for 2 weeks and then given chlorzoxazone (CZX), a procarcinogen and muscle relaxer. Following 24 hours, excretion of its metabolite 6-OH

hydroxychlorzoxazone (6-OHCZX) was reduced 15% in AGE-treated models and 27% in DADS-treated models. Thus, AGE and DADS were found to suppress CYP 2E1 activity.

Potential Mechanisms

Gwilt P, Lear CL, et al. 1994. Cancer Epidemiol Biomarkers Prev. 3(2):155-60.

The metabolism of acetaminophen is very similar to that of carcinogens. According to Gwilt et al., AGE showed only slight increased sulfate conjugation and glucuronide formation suggesting that it inhibits carcinogenesis through a mechanism other than modification of drug metabolism.

Wargovich MJ. 1998. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. Nov 15-17, p. 32.

Using two model systems, one that induces esophageal cancer and the other, colon cancer, it was discovered that diallyl sulfide (DAS) potentially inhibit carcinogenesis. In the aberrant crypt model, DAS and other organosulfur compounds were only effective during initation, not during promotional phase of carcinogenesis, despite being relatively strong inducers of detoxification enzymes, such as glutathione *S*-transferase (GST). Many of the garlic organosulfur compounds are inhibitors of cytochrome P450 2E1 (CYP2E1), the enzymes responsible for the metabolic activation of nitrosomethylbenzylamine and azoxymethane (AOM), two carcinogens used to induce esophageal cancer and colon cancer. Recent work suggests that CYP2E1 gene expression levels are sharply reduced in the presence of DAS and work in progress is aimed at understanding whether this is a unique or common mechanism for cancer chemoprevention by garlic constituents.

Yang CH, Chhabra SK, et al. 2001. J Nutr. 131(3 Suppl):1041S-5S.

Diallyl disulfide (DAS), a constituent found in small amounts in AGE, is metabolically converted to diallyl sulfoxide and diallyl sulfone ($DASO_2$), in which all these compounds are competitive inhibitors of cytochrome P450 2E1 (CYP2E1). $DASO_2$ is also a suicide inhibitor of CYP2E1. Therefore, these compounds are expected to prevent toxicity induced by many environmental chemicals, which are metabolically activated by CYP2E1, in which has been demonstrated with carbon tetrachloride and *N*-nitrosodimethylamine-induced toxicity. DAS, $DASO_2$ and fresh garlic homogenates also inhibited hepatotoxicity caused by a high dose of acetaminophen (APAP), a commonly used analgesic. The protective effect was observed when these events were given before, together with, or within an hour of APAP application. DAS and $DASO_2$ also inhibited the bioactivation of a strong tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and related lung tumorogenesis. DAS and $DASO_2$ also has other biological effects such as the induction of certain CYP enzymes that catalyze the activation of NNK while DAS induced certain CYP enzymes and phase II drug metabolism enzymes and the decrease of hepatic catalase activities. All these effects were observed at concentrations much higher than those resulted from dietary garlic consumption or supplementation.

Cope K, Seifried H, et al. 2009. Anal Biochem. 394(2):243-8.

A new method for extraction and sensitive detection of both *N*-nitrosoproline (NPRO) and *N*-acetyl-*S*-allylcysteine from urine was presented. NPRO excretion has been used as an index for endogenous nitrosation. Urine samples from a study were analyzed to test whether garlic supplementation inhibits NPRO synthesis. Using the method, NPRO and *N*-acetyl-*S*-allylcysteine were quantified and detected in urine. The results suggest that 3 to 5 g of garlic supplements inhibited NPRO synthesis to an extent similar to a 0.5g dose of ascorbic acid or a commercial supplement of AGE. Urinary NPRO concentration was inversely associated with the *N*-acetyl-*S*-allylcysteine concentration.

Inhibited Both the Formation and Bioactivation of Carcinogenic Nitrosamines

Dion ME, Milner JA. 1996. FASEB J. 10(3):A498. Abst #2869.

S-allylcysteine (SAC) was found to inhibit both the formation and bioactivation of the liver carcinogen nitrosomorpholine (NMOR). Adding SAC to a solution of sodium nitrite and morpholine prevented thse two compounds from generating NMOR and also prevented NMOR's ability to mutate a cell model.

Milner JA, Schaffer EM, et al. 1996. 2nd International Congress on Phytomedicine. Munich, Germany. Sep 11-14. Abst #SL-110.

Milner et al. found that *S*-allylcysteine (SAC), diallyl sulfide (DAS) and diallyl disulfide (DADS) were more effective than isomolar ascorbic acid in reducing nitrosomorpholine (NMOR) mutagenicity or ability to damage the DNA of cells.

Sustains Levels of Antioxidants Depleted by a Carcinogen

Balasenthil S, Nagini S. 2000. J Biochem Med Biol Biophys. 4:35-9.

7,12-Dimethylbenz(a)anthracene (DMBA) enhances lipid peroxidation in the circulation of tumor-bearing models. In addition, it significantly depletes circulating antioxidants such as ascorbic acid, vitamin E, reduced glutathione and glutathione peroxidase. Administration of *S*-allylcysteine (SAC) significantly decreased lipid peroxidation and enhanced the levels of antioxidants in models exposed to DMBA-induced oxidative stress.

Borek C. 2001. 2nd International Congerence of the Society for Free Radical Research-Africa. University of Mauritius, Africa. Jul 15-19, p. 49.

An array of dietary antioxidants was reviewed for cancer prevention, including AGE. AGE does not contain the oxidizing allicin, which converts to antioxidants with limited bioavailability, like other forms of garlic. AGE exerts various protective antioxidants actions from scavenging reactive oxygen species to enhancing internal antioxidants in the body, thus preventing free radical damage to DNA, and shows various anticancer activities.

Pinto JT, Lapsia S, et al. 2000. In: Nutrition and Cancer Prevention: New Insights into the Role of Phytochemicals, Advances in Experimental Medicine and Biology. Kluwer Academic Publishers. Vol. 492, pp. 83-106.

Pinto et al. detailed numerous mechanisms whereby at least 20 constituents, including *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC), in garlic, especially AGE, reduce cancer risk. They are effective at each stage of cancer and show such effects at inhibiting uptake and formation of carcinogens, inactivating carcinogens, inhibiting genetic damage, increasing metabolic detoxification, protecting against oxidative damage, restoring normal cell growth/differentiation, stimulating immune surveillance and inhibiting angiogenesis (blood supply to the cancer). They function generally by the following three ways: 1) impeding generation of carcinogens from their precursors, 2) preventing carcinogens from reacting with vulnerable cellular targets, and 3) delaying or reversing expression of malignancy or preventing proliferation of tumor cells.

Inhibited the Growth of Colorectal Carcinoma Cells and Their Angiogenesis

Matsuura N, Miyamae Y, et al. 2006. J Nutr. 136(3 Suppl):842S-6S.

AGE was shown to have antiproliferative effects on colorectal carcinoma cells and inhibitory activity on angiogenesis through the suppression of endothelial cell motility, proliferation and tube formation.

Li S, Yang G, et al. 2017. Oncol Rep. 38(3):1637-44.

The xenografting HCT-116 cancer cells in models, the combination therapy of rapamycin and *S*-allylmercaptocysteine (SAMC) had enhanced tumor-suppressing ability with the upregulation of the Bax/Bcl-2 ratio as a consequence of activated apoptosis, inhibition of autophagic activity and prevention of Akt phosphorylation. The rapamycin and SAMC combination activated antioxidant transcription expressions of nuclear factor erythroid 2-related factor 2 (Nrf2) and downstream gene NAD(P)H:quinine oxidoreductase 1 (NQO1). Cocomitantly, autophagosome cargo p62 was downregulated.

Inhibited the Growth of Colorectal Carcinoma Cells and Their Angiogenesis in Clinical Studies

Ishikawa H. 2002. Jpn J Cancer Chemother. 29(4):545-9.

Ishikawa et al. established a protocol for a randomized controlled trial for prevention of colorectal cancer. The study will include 100 patients with hereditary non-polyposis colorectal cancer who will consume either AGE or placebo, both in capsule form. The main end point of this trial is the number and size of colorectal adenomas after 2 years. Subject recruitment began in March 2002 and the trial will be completed in September 2006.

Tanaka S, Haruma K, et al. 2004. Hiroshima J Med Sc. 53(3-4):39-45.

In a double-blinded randomized study, 51 subjects diagnosed with colorectal adenomas by colonoscopy, were divided into two groups. One group received high-AGE dose (2.4 ml/day) and the other group received low-AGE dose (0.16 ml/day). Out of the 37 subjects chosen for evaluation, there was a 50% decrease rate of at least one adenoma in the high-AGE group 6 to 12 months after intake. There was no decrease in adenomas found in the low-AGE group ($p=0.02$). This study also found that the total size of adenomas increased in the low-AGE group while the increase of adenomas was suppressed in the high-AGE group after 6 to 12 months of intake ($p=0.04$).

Tanaka S, Haruma K, et al. 2006. J Nutr. 136(3 Suppl):821S-6S.

In a preliminary, double-blind, randomized clinical trial, a high dose (2.4 ml/day) and low dose (0.16 ml/day) of AGE was administered to patients with colorectal adenomas for 12 months. Following the 12 months, there was a significant suppression on both the size and number of colon adenomas in patients of the high-dose treatment whereas the number of adenomas increased in the control group. These results suggest that AGE has a suppressive effect on the progression of colorectal adenomas in humans.

Increased Natural-Killer Cells and Cell Activity

Ishikawa H, Saeki T, et al. 2006. J Nutr. 136(3 Suppl):816S-20S.

In a randomized double-blind trial, AGE was administered to patients with inoperable colorectal, liver or pancreatic cancer. It was found that both the number of natural-killer (NK) cells and the NK cell activity increased significantly in the AGE group.

Inhibited the Development of Liver Cancer

Takata N, Yano Y, et al. 1996. 1st International Conference on Gastroenterological Carcinogenesis. Hiroshima, Japan. Pct 22-24. Abst #271.

Takata N, Yano Y, et al. 1997. Jpn J Cancer Res. 88(5):435-42.

S-methylcysteine (SMC) and cysteine was shown to inhibit both the initiation and promotion stages of hepatocarcinogenesis. Suppression of polyamine metabolism and the transitory down-regulation of induction of the proto-oncogene c-jun expression may play important roles in chemopreventive action.

Hori T, Wei M, et al. 1997. 56th Annual Meeting on Japanese Cancer Association. Kyoto, Japan. Sep 26. Abst #CCO-08.

The effects of *S*-methylcysteine (SMC) and cysteine on the development of glutathione *S*-transferase pacental form (GST-P)-positive foci were determined using a diethylnitrosamine-induced hepatocarcinogenesis model system. Both SMC and cysteine significantly inhibited GST-P positive hepatocellular foci. These findings indicate that SMC and cysteine inhibited the development of putative preneoplastic lesions in hepatocarcinogenesis.

Fukushima S. 2000. 6th Annual Meeting of Jpn Mibyou System Assoc. Hiroshima, Japan. Jan 28, p. 45.

Uda N, Kyo E, et al. 2001. 16th Annual Meeting of the Japanese Cancer Association. Yokohama, Japan. Sep 26-28. Abst #2256.

The effect of AGE on the development of glutathione *S*-transferase pacental form (GST-P)-positive foci was determined using diethylnitrosamine-induced hepatocarcinogenesis model system. AGE significantly inhibited GST-P positive hepatocellular foci in a dose-dependent manner. These findings indicate that AGE inhibited the development of putative preneoplastic lesions in hepatocarcinogenesis along with reducing the rate of proliferation of liver cells after partial hepatectomy.

Uda N, Kashimoto N, et al. 2006. J Nutr. 136(3 Suppl):855S-8S.

AGE inhibited the development of putative preneoplastic lesions by hepatocarcinogenesis through treating models with AGE 2, 5, 10 ml/kg intragastrically, 5 times per week, where GST-P-positive foci were significantly decreased. Also, AGE slowed down the proliferation rate of liver cells after partial hepatectomy.

Belloir C, Singh V, et al. 2006. Food Chem Toxicol. 44(6):827-34.

S-allyl cysteine (SAC) and allyl mercaptan (AM) significantly decreased DNA breaks in human hepatocellular carcinoma (HepG2) cells treated with dimethylnitrosamine. Additionally, all the garlic organosulfur compounds studied were shown to decrease the genotoxicity of the direct-acting compounds, hydrogen peroxide and methyl methanesulfonate. This study demonstrated that garlic organic sulfur compounds (OSC) displayed antigenotoxic activity in human metabolically competent cells.

Ng KT, Guo DY, et al. 2012. PloS One. 7(2):e31655.

The proliferation rate and colony-forming abilities of a metastatic hepatocellular carcinoma (HCC) cell line (MHCC97L) cells were suppressed by *S*-allyl cysteine (SAC) together with significant suppression of the expression of the proliferation markers, antigen Ki-67 and proliferating cell nuclear antigen (PCNA). Moreover, SAC hindered the migration and invasion of MHCC97L cells corresponding with up-regulation of E-cadherin and down-regulation of vascular endothelial growth factor (VEGF). Furthermore, SAC significantly reduced apoptosis and necrosis of MHCC97L cells through suppressing B-cell lymphoma-extra large (Bcl-xL) and B-cell lymphoma 2 (Bcl-2) as well as activating caspase-3 and caspase-9.

Pathak S, Catanzaro R, et al. 2018. Drug Chem Toxicol. 12:1-14.

Two liver carcinogens, p-dimethylaminoazobenzene and phenobarbital, were chronically fed to models to produce hepatotoxicity. In the group given AGE daily, down-regulation of B-cell lymphoma 2 (Bcl-2) and tumor protein 53 (p53) caused cell cycle arrest and apoptosis and exhibited additional benefits by arresting cell viability of cancer cells.

Chatterjee S, Patra D, et al. 2019. Environ Toxicol. 34(8):928-40.

S-allyl cysteine (SAC) treatment demonstrated detailed molecular bagatelle associated with p38MAPK mediated effective suppression of cell growth both in HepG2 and chemically induced liver carcinoma. This study suggested significant contribution of p38MAPK-p53-DISC-Caspase pathway in the regulation of anti-neoplastic activity of SAC against hepatocellular carcinoma.

Apoptosis of Gastric Cancer Cells

Lee Y, Kim H, et al. 2011. Biol Pharm Bull. 34(5):677-81.

S-allylmercaptocysteine (SAMC) was found to induce apoptosis in gastric cancer cells *in vitro*. SAMC was reported to inhibit tumor growth rate by 31.36% and 37.78% at doses of 100 and 300 mg/kg, respectively. The apoptosis index of 100 mg/kg and 300 mg/kg of SAMC was 20.87 ± 2.50% and 30.61 ± 2.42%, respectively. For control, 100 mg/kg SAMC and 300 mg/kg SAMC, the positive rate of B-cell lymphoma 2 (bcl-2) protein expression were 15.20 ± 1.67%, 10.94 ± 1.57% and 8.24 ± 1.07% and the positive rate of bax protein expression were 15.30 ± 1.90%, 23.18 ± 1.81% and 25.26 ± 3.03%, respectively. Decreases in bcl-2 messenger RNA (mRNA) and increases in bax mRNA by SAMC in a

dose-dependent manner by reverse transcription-polymerase chain reaction (RT-PCR).

Yan JY, Tian FM, et al. 2013. Eur Rev Med Pharmacol Sci. 17(6):745-51.

Human Gastric Cancer Cells Line (SGC 7901) was cultured with different concentrations of the garlic derived compound *S*-allylmercaptocysteine (SAMC). SAMC at 300 μM induced SGC 7901 apoptosis through cell viability. Polymerase chain reaction (PCR) assay demonstrated that JNK and P38 pathway played an important role.

Zhu X, Jiang X, et al. 2017. Biochem Biophys Res Commun. 491(3):821-6.

Human gastric cancer SGC-7901 cells were inoculated subcutaneously in models. When xenograft tumors reached about 100 mm^3, models were treated with *S*-allylmercaptocysteine (SAMC) for 30 days. SAMC administration effectively delayed the growth of of SGC-7901 xenografts withouts signs of toxicity. TUNEL staining confirmed that the tumors from SAMC-treated models exhibited a markedly higher apoptotic index.

Reduced Risk for Gastric Cancer Death

Li WQ, Zhang JY, et al. 2019. BMJ. 366:l5016.

A group of residents in a county in Shandong province in China with participated in a blinded, randomized, placebo-controlled trial. 2258 participants seropositive for antibodies to *H. pylori* were randomly assigned to *H. pylori* treatment (amoxicillin and omeprazole), vitamin supplementation (vitamins C and E and selenium), garlic supplementation (AGE and garlic oil), or their placebos in a 2x2x2 factorial design, and 1107 *H. pylori* seronegative participants were randomly assigned to vitamin supplementation, garlic supplementation, or their placebos in a 2x2 factorial design. *H. pylori* treatment for 2 weeks and vitamin supplementation and garlic supplementation for 7.3 years were associated with a statistically significant reduced risk of death due to gastric cancer for more than 22 years.

Reduced the Side Effects of Drugs

Yuncu M, Eralp A, et al. 2006. Phytother Res. 20(6):504-10.

AGE when taken along with methotrexate (MTX), a chemotherapy drug, was able to protect the intestine not only on a physiological and pharmacological level, but also at the cellular level. The mechanism for this protection, however, remained to be discovered.

Horie T, Li T, et al. 2006. J Nutr. 136(3 Suppl):861S-3S.

AGE (0.5%) inhibits methotrexate (MTX)-induced apoptosis of muroid intestinal epithelial (IEC-6) cells. These results indicate that AGE may be useful for cancer chemotherapy by reducing the intestinal damage induced by anti-tumor drugs.

Li T, Ito K, et al. 2009. Cancer Chemother Pharmacol. 63(5):873-80.

Methotrexate (MTX)-induced loss of viable muroid intestinal epithelial (IEC-6) cells was almost completely prevented by the presence of more than 0.1% AGE. In IEC-6 cells exposed to MTX, chromatin condensation, DNA fragmentation, caspase-3 activation and cytochrome *c* release and intracellular glutathione (GSH) were preserved to the control levels by the presence of AGE. IEC-6 cells in Gap 2/mitosis (G2/M) stage markedly decreased 72 hours after MTX treatment, was also preserved to the control level by the presence of AGE, indicating that AGE depressed MTX-induced apoptosis of IEC-6 cells, which may be useful for the cancer chemotherapy.

Abdi SA, Najmi AK, et al. 2016. Basic Clin Pharmacol Toxicol. 119(6):598-603.

Cyclophosphamide (CP) is the alkylating anticancer drug that induces a number of toxic effects including hemorrhagic cystitis (HC) in the urinary bladder. Uroplakins are unique urinary transmembrane proteins of urothelium, which may become potential targets of CP metabolites and reactive free radicals. *S*-allyl cysteine (SAC) showed significant ($p<0.001$) protective effects against CP-induced alteration in mRNA levels and protein expression of uroplakin II and protected models from CP-induced HC. SAC was found to be more efficacious in affording protection in urinary bladder tissues than the thiol-rich drug mercaptoethane sulfonic acid (mesna).

Exerts Cytotoxic Effects on Cancer Cells by Altering Mitochondrial Permeability

Ohkubo S, Dalla Via L, et al. 2018. Int J Oncol. 53(3):1257-68.

AGE exerts cytotoxic effects on cancer cells by altering mitochondrial permeability, specifically by activating K+/H+ exchanger in the mitochondria, causing oxidative stress and inducing mitochondrial permability transition (MPT).

Dausch JG, Nixon DW. 1990. Prev Med. 19(3):346-61.

Investigation of garlic studies reveals the many beneficial properties attributed to garlic and its constituents. The sulfur and thiol components have long been examined for their protective effects, such as inhibiting nucleotoxicity in the colon, enhancing the body's mechanism for eliminating exogenous substances/carcinogens and altering Phase 1 and Phase 2 enzymes. Not all garlic processing allows for these beneficial compounds to surface, however, since heat (~60°C) can destroy them. In contrast, AGE can help keep these compounds intact to be fully absorbed.

Nishino H. 1996. Shinyaku to Rinsho (New Drug Clin). 45:451-5.

Since 1981, cancer has been the number one cause of death in Japan and it is speculated that these numbers will continue to rise in the future. Cancer prevention studies using food from all over the world, is one of the most important topics researchers focus on. Recently, the National Cancer Institute (NCI) has presented a designer food program for cancer prevention and garlic is ranked at the top of the important foods. In this review, recent studies on anti-carcinogenesis and anti-tumor properties of garlic and its constituents using various model systems were introduced.

Milner JA. 2006. J Nutr. 136(3 Suppl):827S-31S.

Many evidence points to the anticancer properties of garlic, especially AGE, and a number of specific sulfur compounds from garlic. These prevention characteristics arise through both a dose and temporal related change in several cellular events including those involving cancer-causing chemicals' metabolism, immune system, cell control, and blood supply to the cancer cells. Garlic and its chemical compounds have many mechanisms to inhibit the growth of cancer cells. But there are differences in the efficacy amount these various compounds and across tumor types. Our genetic background may influence such differences. Additional studies are needed with more modest exposures and over prolonged periods for these clarifications. Finally, additional research is needed to identify sensitive "effect" and "susceptibility" biomarkers that can ultimately be used to identify responders from non-responders.

Rivlin R. 2007. 234th ACS National Meeting. Boston, MA. Aug 19-23, p. 17.

Extracts of garlic inhibit human breast, colon and prostate cancer in cell-free systems and in tumors transplanted to models. In early stage androgen-responsive human prostate cancer, garlic compounds accelerate disappearance of testosterone. At later stages, garlic regulates signal transduction and other intracellular processes. Supporting results come from epidemiological reports that increased garlic consumption relates to reduced incidence of prostate cancer. Current studies are determining which garlic derivatives are most active and bioavailable. Future advances will require close collaboration of plant scientists with their medical colleagues to identify and evaluate new agents for disease prevention.

Devrim E, Durak I. 2007. Mol Nutr Food Res. 51(11):1319-23.

Garlic and its preparations have been used for treatment of prostate cancer and relief of benign prostatic hyperplasia (BPH) symptoms for decades. It is thought that the mechanism(s) through which garlic may show anti-cancer and anti-inflammatory effects should be investigated further. Several researchers are attempting to demonstrate the useful properties of garlic and its mechanism(s) of action. This review aims to present the current studies related with the effects of garlic in prostate diseases, namely prostate cancer and BPH.

Ngo SN, Williams DB, et al. 2007. J Nutr. 137(10):2264-9.

Colorectal cancer (CRC) is the third leading cause of cancer death in the United States and the second leading cause of cancer death in Australia. Environmental factors play important roles in the multiple-stage process of CRC and nutritional intervention has been identified as playing a major role in its prevention. All studies conducted over the last decade that examined the effects of garlic on CRC were systematically reviewed. Levels of evidence were ranked from level I to level V according to study designs and the quality of of each study was assessed against a set of quality criteria based on those used by the National Health and Medical Research Council in Australia. One randomized controlled trial (level II) reported a statistically significant 29% reduction in both the size and number of colon adenomas in CRC patients taking AGE (Tanaka et al. 2004). A published meta-analysis (level III) of 7 of these studies confirmed this inverse association, with a 30% reduction in relative risk. Eleven model studies (level V) demonstrated a significant anticarcinogenic effect of garlic and/or its active constituents.

Miroddi M, Calapai F, et al. 2011. Mini Rev Med Chem. 11(6):461-72.

Literature data on the effects of garlic and garlic compounds which can serve as basic information to design clinical approach in oncohematology were analyzed. Oil-soluble sulfur compounds are responsible for anticancer effects exerted through multiple mechanisms such as inhibition of metabolic carcinogenic activation, arrest of cell cycle, antioxidant and pre-apoptotic action. Evidence about the effects of diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), ajoene and *S*-allyl mercaptocysteine (SAMC) in oncohematology was described. This research highlights that data on garlic in oncohematology are essentially represented by pre-clinical studies, which provided insight into biological activities of garlic compounds and support a rationale for the use of DAS, DADS, DATS and ajoene as

promising agents in oncohematology.

Agbana YL, Ni Y, et al. 2019. Nutr Res. 73:1-14.

Some potential mechanisms responsible for the anticarcinogenic action of S-allylcysteine (SAC) were suggested: induction of carcinogen detoxification, inhibition of cell proliferation and growth, mediation of cell cycle arrest, induction of cell death, inhibition of epithelial-mesenchymal transition and cell invasion, suppression of metastasis, and induction of immunomodulation in cancer cells. More specific studies, specifically clinical and epidemiological, are required to advance the promising use of SAC as a chemopreventive and therapeutic agent in cancer.

Lv Y, So KF, et al. 2019. Chin J Nat Med. 17(1):43-9.

The up-to-date mechanistic pathways associated with the anti-proliferative, anti-metastatic and pro-apoptotic effects of *S*-allylmercaptocysteine (SAMC) in various cancer models are discussed in this review. SAMC as gained recognition as a promising daily food supplement for cancer prevention or management.

Overview of Inhibition of Cancer Growth by AGE and its Constituents		
Cancer	***In vivo* (model)**	***In vitro* (cell culture)**
Bladder	Model[1-5]	
Breast	Model[9-23]	Human[6-8]
Colon	Model[24-30]	Human[31]
Erythroleukemia		Human[32,33]
Esophagus	Model[34]	
Liver		Model[35-37]
Lung	Model[38-40]	Human[51]
Melanoma		Human[41,42], Model[42]
Neuroblastoma		Human[43]
Prostate		Human[44-46]
Skin	Model[47-50]	
Stomach	Model[38-40]	

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Improved Survival, Memory Retention, Learning Deficits and Immune Response

Zhang YX, Saito H, et al. 1994. In: The SAM Model of Senescence. Elsevier Science B.V., pp. 451-4.

The thymus plays a key role in balancing the nervous and immune systems (neuroendocrine immunomodulation network or NIM). Thymectomy, or removal of the thymus gland, reduces immune function and causes an imbalance in NIM, further accelerating the aging process. AGE was found to significantly improve the impairment in memory induced by thymectomy in senescence-accelerated prone 8 (SAMP8) models. A trend toward improved learning ability and immune function by improving the number of plaque-forming splenocytes was also noted suggesting the anti-aging effects of AGE may be due to improvement of immune function.

Moriguchi T, Saito H, et al. 1994. The SAM Model of Senescence. Elsevier Science B.V., pp. 447-50.

AGE was found to delay the manifestation of learning and memory impairments in senescence-accelerated prone 8 (SAMP8) models. Furthermore, regular ingestion of AGE significantly improved the learning performance of normal senescence-accelerated-resistant 1 (SAMR1) models. AGE improved the survival of SAMP8 models by restraining their aging speed via multiple biological mechanisms such as antioxidation and immunomodulation.

Moriguchi T, Saito H, et al. 1994. Biol Pharm Bull. 17(12):1589-94.

In another study, AGE was found to increase the survival ratio of senescence-accelerated models and enhance their memory retention as indicated by various memory tests. It was suggested that AGE might be useful for treating physiological aging and age-related memory deficits in humans.

Moriguchi T, Saito H, et al. 1996. Biol Pharm Bull. 19(2):305-7.

A diet containing 2% AGE extended the survival ratio of a strain of senescence-accelerated prone 8 (SAMP8) models to that of a senescence-accelerated resistant 1 (SAMR1) models. Moreover, AGE markedly improved learning deficits in the SAMP8 models.

Nishiyama N, Moriguchi T, et al. 1996. Int Acad Biomed Drug Res. 11:253-8.

Nishyama N, Moriguchi T, et al. 1997. Exp Gerontol. 32(1-2):149-60.

AGE supplementation improved the survival of senescence-accelerated prone 8 (SAMP8) models to that of senescence-accelerated resistant 1 (SAMR1) that are not prone to accelerated aging. AGE decreased the total number of errors in two different passive avoidance tests showing an improvement in learning/memory.

Nishiyama N, Moriguchi T, et al. 1996. Int Acad Biomed Drug Res. Basel Karger. 11:253-8.

Moriguchi T, Nishiyama N, et al. 1996. Phytother Res. 10:468-72.

AGE's F-4 protein fraction markedly increased the survival of cultured hippocampal neurons, the first clear evidence that AGE interacts with brain neurons.

Satio H, Nishiyama N. 1996. 2nd International Congress on Phytomedicine. Munich, Germany. Sep 11-14. Abst #SL-94.

AGE was shown to improve memory acquisition and memory retention processes in avoidance tasks. Also, AGE increased survival of cultured hippocampal neurons and the number of branching points per axon, showing that AGE has beneficial effects on aging and contains multiple components with neurotrophic activity.

Moriguchi T, Saito H, et al. 1996. 116th Annual Meeting of the Pharmaceutical Society of Japan. Kanazawa, Japan. Mar 27-29. Abst #27-J1-10-1.

Moriguchi et al. sought to evaluate the effect of AGE using a model system with memory loss and learning impairments. Reduced brain weight and atrophy of the forebrain caused models to become learning impaired and experience memory loss. However, significant improvements were seen with AGE supplementation.

Zhang Y, Moriguchi T, et al. 1997. Ch. 13. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 117-29.

Chronic oral administration of AGE was found to significantly ameliorate both thymectomy-reduced antibody production response and thymectomy-induced impairment of learning behaviors in two strains of models. AGE also restored the significantly increased levels of hypothalamic norepinephrine 3,4-dihydroxyphenylacetic acid and homovanilic acid, and the hypothalamic choline acetyltransferase activity to control levels. Chronic ingestion of AGE significantly potentiated lymphocyte proliferation induced by concanavalin A or lipopolysaccharides in both senescence-accelerated prone 8 (SAMP8) and senescence-accelerated resistant 1 (SAMR1) models, suggesting that AGE ameliorated age-related deterioration of learning and memory ability and immune response.

Moriguchi T, Saito H, et al. 1997. Clin Exp Pharmacol Physiol. 34(3-4):235-42.

At 10 months of age, the grading score for aging (with higher number being the most aged) for age-accelerated models (senescence-accelerated prone 10 or SAMP10) was significantly higher than that of senescence-accelerated resistant 1 (SAMR1) models. Administration of AGE prevented the increase in the

grading score of SAMP10 and SAMR1. AGE also improved learning and memory deficits of SAMP10 in both passive and conditioned avoidance tests and in spatial memory tests.

Nishiyama N. 2000. 6th Annual Meeting of Jpn Mibyou System Assoc. Hiroshima, Japan. Jan 28, p. 44.

Immune function disorders may cause a change in the central nervous system through the immune-endocrine-nerve network. Senescence-accelerated models caused a reduction in spleen proliferation and antibody production. However, AGE supplementation inhibited these reductions. AGE also improved the immunoresponse, noradrenaline content and learning impairment. Therefore, long-term supplementation with AGE may be associated with memory and learning improvement through enhanced immune function.

Rahman K. 2003. Ageing Res Rev. 2(1):39-56.

In this study, Rahman proposed that the elderly can benefit from taking garlic, either for preventing or reducing chronic diseases associated with old age. The premise associated with this effect is due to the potent antioxidant properties of AGE.

Kim JM, Chang N, et al. 2006. Biosci Biotechnol Biochem. 70(8):1969-71.

S-allyl cysteine (SAC), an active organosulfur compound derived from garlic, was found to reduce mortality with lesser incidence of stroke and also to lower overall stroke-related behavioral score in spontaneously hypertensive stroke prone (SHRSP) models by dietary administration.

Imai T, Kosuge Y, et al. 2007. Neuroscience. 147(3):639-51.

Amyloid β protein (Aβ) potentiation of tunicamycin-induced neurotoxicity was reversibly blocked by *S*-allyl cysteine (SAC) and L-type calcium channel blocker nifedipine, in a restricted neuronal area of the muroid organotypic hippocampal slice cultures (OHCs). SAC, when simultaneously applied, also reversed the increases in calpain activity and the active forms of caspase-12 and caspase-13 by Aβ+TM with no change in the increased levels of glucose regulated protein (GRP)94, GRP78 and cytidine-cytidine-adenosine-adenosine-thymidine (CCAAT)/enhancer binding protein (C/EBP) homologous protein (CHOP). These results indicate that Aβ facilitates the calpain-caspase-12-caspase-13 pathway, thus potentiating TM-induced neuronal death in the hippocampus.

Ishige K, Takagi N, et al. 2007. J Pharmacol Sci. 104(1):46-55.

Organotypic hippocampal slice cultures (OHCs) were cultured for 7 weeks, *S*-allyl cysteine (SAC) protected the cells in *Comu Ammonis* area 1 (CA1) and CA3 and the dentate gyrus from amyloid-β $(Aβ)_{25-35}$-induced toxicity. The increases in cleaved caspase-12 were also reversed by simultaneously applied SAC, suggesting that OHCs cultured for relatively longer periods are more susceptible to Aβ-induced toxicity and that the Aβ-induced cell death involves caspase-12-dependent pathways and SAC is able to protect against the Aβ-induced neuronal cell death through inhibition of the caspase-12-dependent pathway.

Elinos-Calderon D, Robledo-Arratia Y, et al. 2010. J Neural Transm. 117(1):35-44.

S-allyl cysteine (SAC) was tested as a post-treatment in different *in vitro* and *in vivo* models. Quinolinic acid (QUIN) was used as a typical excitotoxic/pro-oxidant inducer, 3-nitropropionic acid (3-NP) was employed as a mitochondrial function inhibitor, and their combination (QUIN + 3-NP) was also evaluated in *in vitro* studies. For *in vitro* purposes, concentrations of SAC were added to isolated brain synaptosomes at different times after the incubation with toxins. For *in vivo* studies, SAC was given to QUIN- or 3-NP-strially lesioned models for 7 consecutive days. A differential pattern of protection was achieved by SAC, mostly expressed in the 3-NP toxic model, in which nerve ending protection was found within the first hour after the toxic insult started.

Garcia E, Villeda-Hernandez J, et al. 2010. Phytomedicine. 18(1):65-73.

Treatment of models with *S*-allyl cysteine (SAC) for 5 days in parallel to 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridinium (MPTP) significantly reduced the degree of cell damage and prevented immunoreactivities of glial fibrillary acidic protein (GFAP), tumor necrosis factor-α (TNF-α) and inducible nitric oxide synthase (iNOS), as well as a reduced number of apoptotic nuclei. These results suggest that MPTP-induced morphological alterations recruit a pro-inflammatory component triggered by cytokine TNF-α release and nitric oxide formation, which is sensitive to the antioxidant properties of SAC. SAC is an effective experimental tool to reduce the brain lesions associated with oxidative damage and inflammatory responses.

Javed H, Khan MM, et al. 2011. Brain Res. 1389:133-42.

Models pre-treated with *S*-allyl cysteine (SAC) (30 mg/kg) and vehicle (intraperitoneal; once daily for 15 days) were bilaterally injected with intracerebroventricular streptozotocin (ICV-STZ), whereas sham models received the same volume of vehicle. SAC pre-treatment prevented the cognitive and neurobehavioral impairments. Significant protection against an increased latency and path length observed in lesion, activities of reduced glutathione (GSH), glutathione peroxidase (GPx) and glutathione reductase (GR) in STZ group pre-treated with SAC. SAC pre-treated group also significantly attenuated the elevated level of thiobarbituric acid reactive substances (TBARS) and protected apoptotic parameters like DNA fragmentation, expression of B-cell lymphoma 2 (Bcl2) and protein 53 (p53).

Rojas P, Serrano-Garcia N, et al. 2011. J Nutr Biochem. 22(10):937-44.

Models were pretreated with *S*-allyl cysteine (SAC) daily for 17 days, followed by administration of 1-methyl-4-phenylpyridinium (MPP^+). Models receiving SAC had significantly attenuated MPP^+-induced loss of striatal dopamine (DA) levels (32%). The neuroprotective effect of SAC against MPP^+ neurotoxicity was associated with blocked (100% of protection) of lipid peroxidation and reduction of superoxide radical production. Behavioral analyses showed that SAC improved MPP^+-induced impairment of locomotion (35%).

Zarezadeh M, Baluchnejadmojarad T, et al. 2017. Eur J Pharmacol. 795:13-21.

S-allyl cysteine (SAC) was administered to models p.o. at doses of 25, 50, or 100 mg/kg/day, 30 minutes after lipopolysaccharide (LPS)-induced cognitive deficit, for seven days. SAC at 100 mg/kg/day improved spatial recognition memory in Y maze, discrimination ratio in novel object discrimination task, and retention and recall in passive avoidance test, mitigated lipid peroxidation marker malondialdehyde (MDA) and augmented superoxide dismutaste (SOD), catalase and glutathione (GSH) in hippocampal homogenate and lowered acetylcholinesterase activity. Meanwhile, SAC down-regulated nuclear factor κ B, toll-like receptor 4 (TLR4), glial fibrillary acidic protein (GFAP), and interleukin 1β (IL-1β) and up-regulated nuclear factor (erythroid-derived 2)-like 2 (Nrf2) in addition to lowering iba1-immunoreactive intensity in the hippocampus of LPS-injected group.

Enhanced Nerve Growth

Zhang YX, Saito H, et al. 1994. In: The SAM Model of Senescence. Elsevier Science B.V., pp. 451-4.

AGE was found to prolong the survival of cultured neurons from embryonic hippocampus *in vitro*, suggesting a direct neurotrophic effect.

Nishiyama N, Moriguchi T, et al. 1996. Int Acad Biomed Drug Res. 11:253-8.

AGE and its F-4 protein fraction markedly increased the survival of cultured hippocampal neurons, providing clear evidence that AGE interacts with brain neurons. AGE also demonstrated a neurotrophic effect by enhancing the axonal branching of nerve endings.

Moriguchi T, Nishiyama N, et al. 1996. Phytother Res. 10:468-72.

Moriguchi et al. confirmed that AGE could significantly prolong the survival of hippocampal neurons and increase the number of branching points per axon when added to the culture medium. The protein fraction from AGE (F-4) also showed a potent survival-promoting effect on these neurons. AGE was concluded to contain multiple components with neurotrophic activity and for this reason may be useful for preventing age-related morphological changes in the central nervous system.

Moriguchi T, Saito H, et al. 1997. Clin Exp Pharmacol Physiol. 34(3-4):235-42.

AGE was found to prevent the decrease in brain weight and the atrophic changes in the frontal brain at 12 months of age in senescence-accelerated prone 10 (SAMP10) models. The authors suggested that AGE prevents physiological aging and may be beneficial to age-related cognitive disorders.

Moriguchi T, Matsuura H, et al. 1997. 2nd International Symposium on Natural Drugs. Maratea, Italy. Sep 28-Oct 1, p. 73.

Low molecular weight chemicals from AGE were found to promote neuronal survival in hippocampal culture studies. Allixin enhanced neuronal survival and axonal branching but was cytotoxic at high dosages. A related compound, 2,6-dimethyl-3-hydroxy-4H-pyrone (DHP), possessed strong neurotrophic activity but was much less toxic. *S*-allyl cysteine (SAC) also enhanced neuronal survival and branching. It was suggested that these compounds in AGE may be helpful in the development of therapeutic and/or prophylactic drugs for neurodegenerative disorders.

Moriguchi T, Matsuura H, et al. 1997. Neurochem Res. 22(12):1449-52.

S-allyl cysteine (SAC), an organosulfur compound in AGE with a thioallyl group, promoted the axonal branching of cultured neurons. SAC also promoted neuronal survival. Γ-glutamyl-*S*-allyl-L-cysteine also exerted neurotrophic activity similar to SAC.

Sumi S, Tsuneyoshi T, et al. 2001. J Nutr. 131(3 Suppl):1096S-9S.

A possible mechanism in which AGE regulates gene expression resulting in neurotrophic effects was found. Four complementary DNA (cDNA) clones designated #24, #110, #153 and #155 were obtained by screening genes differentially expressed by the addition of AGE in primary cultured hippocampal neurons isolated from fetal model brain, using messenger RNA (mRNA) differential display technology. Dot blot hybridization combined with reverse transcription-polymerase chain reaction (RT-PCR) confirmed that the transcription from these four genes was enhanced at least 3-fold. mRNA of #153 increased more than 20 times. The nucleotide sequence of cDNA clone #153 coincided completely with that of the corresponding region of α 2-microglobulin-related protein (α2MRP) gene.

Improved Serotonin Level

Fillion GM, Fillion MP, et al. 1997. Ch. 19. In: Nutraceuticals:

Fillion et al. found that AGE may affect serotonin release in the brain. In cases of impulsivity, depression

Designer Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 189-92.

and such pathologies, the serotonergic 5-hydroxytryptamine (5-HT) system is in deficit. Antidepressants are often given in an attempt to boost the serotonin system. Monoamine oxidase (MAO) inhibitors, for example, block the enzyme that degrades serotonin. Other antidepressants attempt to make more serotonin available. The serotonergic system exerts its effect on assorted neurotransmitters in the central nervous system via a number of different receptors.

Interaction with various receptors controls the release of serotonin. AGE may modulate receptors which bind to serotonin inhibiting its release, thus making more serotonin available. Specifically, AGE enhanced the binding of [3H] 5-HT to 5HT1 receptors, likely 5-HT1B/1D serotonin receptors in a non-competitive manner. By making more serotonin available, AGE may potentially help to alleviate various pathologies resulting from its deficiency as well as enhance the immune system.

Anti-Alzheimer's Effect

Gwebu ET, Griffin B, et al. 1998. Soc Neurosci 28th Annual Meeting. Los Angeles, CA. Nov 7-12. 24(2):Abst #571.11.

Selassie M, Griffin B, et al. 1999. In Vitro Cell Dev Biol Amin. 35(7):369-70.

Griffin B, Selassie M, et al. 2000. Nutr Neurosci. 3:139-42.

Neuronal PC12 proliferating cells were treated with nerve growth factor for 10 days and tested for their viability. B-amyloid$_{25-35}$ (Aβ), a neurotoxin that is a progenitor of oxidative stress. The addition of AGE to the medium protected neuronal PC12 cells against Aβ (70 μg/ml) toxicity in a dose-dependent manner. Since the accumulation of Aβ is a hallmark for Alzheimer's disease, the most common cause of progressive intellectual failure and memory impairment among the elderly, it was suggested that AGE can be useful for the amelioration of this disease.

Peng Q, Buz'Zard AR, et al. 2002. Med Sci Monit. 8(8):BR328-77.

One of the pathological features of Alzheimer's disease (AD) is neuronal apoptosis. This condition is associated with senile plaques containing amyloid-β (Aβ) peptide, which are assumed to be involved with reactive oxygen species (ROS), within the Aβ-mediated neurotoxicity apoptotic mechanism. The researchers treated neuronal PC12 cells with AGE and *S*-allyl cysteine (SAC) for 24 hours prior to exposure to Aβ. Cell viability, DNA fragmentation, number of apoptotic cells, caspase activity and generation of ROS were observed. Conclusions drawn from this study suggest that AGE and SAC reduced cell viability and suppressed Aβ-induced apoptosis in PC12 cells.

Moriguchi T, Saito H. 2002. Piante Medicinali. 1(3):114-28.

Moriguchi et al. reviews the neurotrophic effect of AGE based on preclinical studies. AGE has been shown to possess neurotrophic effects and protect brain or reduce dementia in the Alzheimer model. *S*-allylcysteine (SAC) and other constituents in AGE may be active compounds.

Ito Y, Kosuge Y, et al. 2003. Neurosci Res. 46(1):119-25

Ito Y, Ito M, et al. 2003. Brain Res. 985(1):98-107.

Amyloid β-protein (Aβ) is associated with senile plaques in the brains of Alzheimer's patients and is cytotoxic to cultured neurons. The data from this study indicate that *S*-allyl-L-cysteine (SAC) may have a unique and protective effect on Aβ- and tunicamycin-induced cell death in neurons and other cell lines.

Kosuge Y, Koen Y, et al. 2003. Neurosci 122(4):885-95.

S-allyl-L-cysteine (SAC) has been shown to possess various biological effects, including neurotrophic activity. This study characterizes the neuronal death induced by amyloid β-protein (Aβ), 4-hydroxynonenal (HNE), tunicamycin and trophic factor deprivation, and investigates how SAC could prevent this effect. Results of this study indicate that SAC could protect against the neuronal cell death that is triggered by endoplasmic reticulum (ER) dysfunction in the hippocampus.

Brown C, Gwebu ET. 2003. FASEB J. 17(4):A603. Abst #377.12.

Mbyirukira GB, Gwebu ET. 2003. FASEB J. 17(5):A981. Abst #623.3.

Alzheimer's disease is characterized by the loss of neuronal cells, most likely due to apoptotic death. Apoptosis is induced by the activation of caspase-3 in which studies show that dietary AGE inhibits human recombinant caspase-3. Results of the study present evidence that AGE inhibits cellular caspase-3 activity in neuronal PC12 cells.

Jackson R, McNeil B, et al. 2003. FASEB J. 17(4):A604. Abst #377.17.

Jackson R, McNeil B, et al. 2003. Nutr Neurosci. 5(4):287-90.

AGE was reported to inhibit caspase-3 which may be effective in reducing apoptotic death of neurons, since caspase inhibitors have been shown to inhibit neuronal cell death.

Baker M, Mbyirukira H, et al. 2003. FASEB J. 17(4):A604. Abst #377.18.

S-adenosyl-methionine (SAM), a constituent in AGE, induces apoptosis in neuronal PC12 cells. AGE provides protection from apoptosis, thereby protecting from Alzheimer's disease due to its activity by its SAM compound.

Eilliot D, Jackson R, et al. 2003. FASEB J. 17(4):604. Abst #377.13.

Nitric oxide (NO) is an essential metabolite for normal physiological function, however, at higher

concentrations it can be neurotoxic and perhaps by inducing apoptosis. Results of the study show that AGE protect neuronal PC12 cells from the cytotoxicity of NO donors and may be helpful in the fight against Alzheimer's disease.

Borek C. 2006. J Nutr. 136(3 Suppl):810S-2S.

Evidence suggests that risk factors for cardiovascular disease such as high cholesterol, hypertension, high homocysteine and inflammation, increases the risk of dementia including its most common form, Alzheimer's disease (AD). Clinical studies have shown that AGE may help prevent cardiovascular and cerebrovascular diseases and lower the risk of dementia and AD by inhibiting cholesterol, low-density lipoproteins (LDL) oxidation, platelet aggregation, arterial plaque formation, decreasing homocysteine, lowering blood pressure, increasing microcirculation, protecting neurons from amyloid β-protein (Aβ) neurotoxicity and apoptosis.

Kosuge Y, Sakikubo T, et al. 2006. Neurochem Int. 49(3):285-93.

Hippocampal neurons (HPN) were protected from neuronal cell death in the presence of *S*-allyl cysteine (SAC). In contrast, SAC did not exhibit any protective effects on cerebellar granule neurons (CGN). Both neurons and found in the endoplasmic reticulum (ER) are vulnerable to neuronal cell death with ER stress. Research has shown ER stress to be an important factor in amyloid β-peptide (Aβ)-induced neutotoxicity and Alzheimer's disease pathology.

Chauhan NB. 2006. J Ethnopharmacol. 108(3):385-94.

Chauhan concludes that therapy for Alzheimer's patients may be improved by the many beneficial activities of AGE, e.g., antioxidant, anti-inflammatory and anti-apoptotic effects. In this study, a reduction in cerebral plaques and inflammation, sodium dodecyl sulfate (SDS)-extractable detergent soluble and formic acid extractable detergent resistant β-amyloid (Aβ) species, and tau phosphorylation was seen in models with Alzheimer's disease given AGE. All of which are characteristics found in the pathophysiology of Alzheimer's disease.

Chauhan NB, Sandoval J. 2007. Phytother Res. 21(7):629-40.

Accumulation of β-amyloid (Aβ) oligomers of Aβ42 species, in particular, is known to correlate with cognitive deficits independent of Aβ plaque deposition in the brain. Majority of the research showing behavioral improvement after cerebral Aβ reduction has been reported when the models carried fewer/abundant amyloid plaques in the brain. Feeding of AGE prevented deterioration of hippocampal based memory tasks in models with mild cognitive impairment (MCI) stage of Alzheimer's disease (AD), by slowing plaque development with a predominance of Aβ40.

Ray B, Chauhan NB, et al. 2011. J Neurochem. 117(3):388-402.

Significant neuroprotective and neurorescue properties of AGE and one of its ingredients, *S*-allyl cysteine (SAC), from reactive oxygen species (ROS) hydrogen peroxide (H_2O_2)-mediated insults to neuronal cells were observed. Treatment of AGE and SAC were found to protect neuronal cells when they were independently co-treated with ROS. Furthermore, a novel neuropreservation effect of AGE was detected in that pre-treatment with AGE alone protected ~80% neuronal cells from ROS-mediated damage. AGE was also found to preserve synaptosomal-associated protein 25 (SNAP25) from ROS-mediated insult. For example, treatment with 2% AGE containing diet and SAC (20 mg/kg of diet) independently increased (~70%) levels of SNAP25 and synaptophysin in Alzheimer's amyloid precursor protein-transgenic models, of which the latter was significantly decreased in Alzheimer's disease (AD).

Tsai SJ, Chiu CP, et al. 2011. J Agric Food Chem. 59(11):6319-26.

In models with brain injury induced by D-galactose (DG), *S*-allyl cysteine (SAC), *S*-ethyl cysteine (SEC) and *S*-propyl cysteine (SPC) significantly decreased the production of amyloid-β (Aβ) peptide (1-40) and Aβ (1-42) and suppressed the expression of β-amyloid precursor protein (APP) and β-site APP cleavage enzyme 1 (BACE1) ($P<0.05$). Intake of SAC, SEC and SPC significantly retained protein kinase C (PKC) activity and the expression of PKC-α and PKC-γ ($P<0.05$), significantly lowered aldose reductase (AR) activity, AR expression and carboxymethyllysine (CML) and pentosidine levels ($P<0.05$), and significantly decreased reactive oxygen species (ROS) and protein carbonyl levels and restored brain glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase activities ($P<0.05$). These findings support that SAC, SEC and SPC via their anti-Aβ, antiglycative and antioxidative effects are potent agents against the progression of neurodegenerative disorders such as Alzheimer's disease.

Denzer I, Münch G, et al. 2016. Food Chem. 194:843-8.

Preincubation with *S*-allyl-l-cysteine (SAC) and isoliquiritigenin increased mitochondrial membrane potential (MMP) in both pheochromocytoma cell (PC12) cell models of oxidative and nitrosative stress in a similar range as the positive control l-sulforaphane.

Manral A, Meena P, et al. 2016. Neurotox Res. 30(3):407-26.

Diallyl disulfide (DADS) analogues 7k and 7l significantly inhibited Aβ1-42-induced neuronal cell death by inhibiting reactive oxygen species (ROS) generation in SH-Sy5Y human neuroblastoma cells. Moreover, they prevented apoptosis, in response to ROS, by restoring normal Bax/Bcl-2 ratio. Furthermore, it was observed that scopolamine-induced memory impairment in a model was coupled by

alterations in neurotransmitters, acetylcholinesterase activity and oxidative stress markers. Administration of 7k and 7l at 5 mg/kg significantly reversed scopolamine-induced behavioral, biochemical, neurochemical and histological changes in a manner comparable to standard donepezil.

Enhancement of Human Growth Hormone

Buz'Zard AR, Peng Q, et al. 2002. Growth Horm IGF Res. 12(1):34-40.

The amount of human growth hormone decreases significantly after age 30. This decrease has been implicated as one of the major causes in the signs of aging, such as thinning of the skin and bones, a decrease in lean muscle mass and an increase in adipose tissue. Supplementing the body's dwindling supply with recombinant human growth hormone (rHGH) has been shown to reverse the signs and symptoms of aging. However, it is quite costly, requires repeated injections and has side effects such as carpel tunnel syndrome, gynecomastia and insulin resistance. Buz'Zard et al. reported that a combination of equal amounts of L-arginine and L-lysine, Kyolic® AGE, *S*-allyl cysteine (SAC) and Pycnogenol® significantly increased the secretions of HGH in an *in vitro* model of genetically-engineered keratinocytes.

Protective Effect in Spinal Cord Injury

Cemil B, Gokce EC, et al. 2012. Ulus Travma Acil Cerrahi Derg.

Models with spinal cord trauma were divided into 3 groups. The models in the AGE group were administered 250 mg/kg AGE per day diluated in tap water orally by gavage for 15 days prior to trauma. Results show decreased malondialdehyde (MDA) levels and increased superoxide dismutase (SOD) levels in the AGE group compared with the spinal cord injury (SCI) group and control group, demonstrating neuroprotective effects.

Cemil B, Gokce EC, et al. 2016. J Med Food. 19(6):601-6.

AGE given to models with spinal cord ischemia-reperfusion (I/R) had significantly higher Basso, Beattie, and Bresnahan (BBB) scores and significantly higher antioxidant enzymes levels, and significantly reduced the inflammatory cytokines and caspase-3 activity than the I/R group. The AGE group showed reduced degree of ischemia and spinal cord edema, pathologically, and preservation of tissue structure. Also, there was significant difference between the sham (no I/R) and AGE groups in terms of total antioxidant enzyme levels.

Prevented Dietary Saturated-Fat Induced Disturbances in Blood-Brain Barrier Function

Takechi R, Pallebage-Gamarallage MM, et al. 2013. J Neuroinflammation. 10:73.

Models were fed a diet enriched in satured fatty acids (SFA) for 9 months to induce systemic inflammation and blood-brain barrier (BBB) disturbances. Nutraceutical agents including AGE were used in the positive control SFA-group and in low-fat fed controls. The nutraceutical agents including AGE completely prevented the SFA-induced disturbances of BBB which include brain parenchymal abundance of immunoglobulin G (IgG) and apolipoprotein B (apoB) lipoproteins with increased parenchymal glial fibrillar acidic protein (GFAP) and cyclooxygenase-2 (COX-2), and normalized the measures of neurovascular inflammation and oxidative stress.

Antioxidant Activity and Its Effect on Cerebral Ischemia

Cervantes MI, de Oca Balderas PM, et al. 2013. Food Chem. 140(1-2):343-52.

Models with cerebral ischemia were administered AGE and 20% hydroethanolic fresh extracts from garlic clove (GCE) and skin (GSE). All three extracts scavenged superoxide anion, peroxynitrite anion, and peroxyl radicals, but with different efficacies. GCE and GSE scavenged hydroxyl radicals and GSE scavenged singlet oxygen, which significantly prevented reduction of neuronal nuclear antigen in the infracted area but with no improvement in neurological function. Extracts decreased mRNA expression of NR1- and NR2B-NMDA-receptor subunits and prevented ischemia-induced reduction in mitochondrial potential and in ATP synthesis, indicating that antioxidants present in three garlic extracts may regulate reactive oxygen species (ROS) concentrations during ischemia, favor pro-survival pathways and attenuate mitochondrial dysfunction.

Gomez CD, Aguilera P, et al. 2019. Adv Clin Exp Med. 28(12):1609-14.

In experimental models of cerebral ischemia, AGE and S-allylcysteine (SAC) treatments increased the main neuronal glucose transporter (GLUT3) and glutamate cysteine ligase catalytic subunit (GSLC) mRNA levels in control and under ischemic/reperfusion injury models. This suggests that AGE and SAC might induce neuroprotection, while controlling reactive oxygen species (ROS) levels, as indicated by the increase in GCLC expression, and regulating the energy content of the cell by increasing glucose transport mediated by GLUT3.

Protect Against Neurotoxicity

Garcia E, Santana-Martinez R, et al. 2014. Free Radic Res. 48(2):159-67.

SAC at 120 mg/kg, i.p., for 5 days in models partially ameliorated the 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridinium (MPTP) (30 mg/kg, i.p., for 5 days)-induced striatal and nigral dopamine and tyrosine hydroxylase depletion, attenuated the loss of Mn-SOD and HO-1 activities, and preserved the protein content of these enzymes. These findings suggest that SAC can exert neuroprotection since the origin of the dopaminergic lesion-at the substantia nigra (SN)-not only by means of direct antioxidant actions, but also through the Nrf2 nuclear transactivation and Phase 2 enzymes upregulation.

Neuroprotection Against Endoplasmic-Reticulum Stress-Induced Neuronal Death

Imai T, Kosuge Y, et al. 2014. Amino Acids. 46(2):385-93.

S-allycysteine (SAC) in AGE reversibly restored the survival of cultured model hippocampal neurons (HPNs) and increased the degradation of α-spectrin, a substrate of calpain, induced by tunicamycin, a typical ER stress inducer. Activities of μ- and m-calpains *in vitro* were also concentration dependently suppressed by SAC. SAC (1 mM) significantly reversed the effect of PD150606 at a concentration that elicited supramaximal inhibition (100 μM), but did not affect ALLN (1 nM)- and calpastatin (100 nM)-induced inhibition of μ-calpain activity. SAC has protective effects against ER stress-induced neuronal cell death attributable to suppression of calpain through interaction with its Ca^{2+}-binding site.

Attenuates Neuroinflammatory Response

Zhou H, Qu Z, et al. 2014. PLoS One. 9(11):e113531.

AGE and N-α-(1-deoxy-D-fructos-1-yl)-L-arginine (FruArg) both were shown to significantly inhibit lipopolysaccharide (LPS)-induced nitric oxide (NO) production in in murine BV-2 microglial cells. Expressions of 26 proteins were significantly altered upon LPS exposure, while levels of 20 and 21 proteins exhibited significant changes in response to AGE and FruArg treatments, respectively, in LPS-stimulated BV-2 cells. Notably, approximately 78% of the proteins responding to AGE and FruArg treatments are in common. These results suggest that AGE and FruArg attenuate neuroinflammatory responses and promote resilience in LPS-activated BV-2 cells by suppressing NO production and by regulating expression of multiple protein targets with oxidative stress.

Song H, Lu Y, et al. 2016. Sci Rep. 6:35323.

Both AGE and N-α-(1-deoxy-D-fructos-1-yl)-L-arginine (FruArg) significantly attenuate lipopolysaccharide (LPS)-induced neuroinflammatory responses in BV-2 microglial cells. AGE reversed 67% of the mRNA alteration induced by LPS, whereas FruArg accounted for the protective effect by reversing expression levels of 55% of genes altered by LPS. Key pro-inflammatory canonical pathways induced by the LPS stimulation were modulated by treatment with both AGE and FruArg.

Zarezadeh M, Baluchnejadmojarad T, et al. 2016. Eur J Pharmacol. 795:13-21.

S-allylcysteine (SAC) mitigated lipopolysaccharide (LPS)-induced cognitive deficit in models via attenuation of oxidative stress, neuroinflammation, astroliosis, and acetylcholinesterase activity.

Zeinali H, Baluchnejadmojarad T, et al. 2017. Biomed Pharmacother. 97:557-63.

S-allylcysteine (SAC) was administered p.o. at a dose of 50 mg/kg/day to models immunized with myelin oligodendrocytic glycoprotein (MOG35-55). Results showed that SAC alleviated clinical signs and severity of multiple sclerosis (MS) and improved lumbar spinal cord tissue of tumor necrosis factor α (TNFα), interleukin 17 (IL-17), activity-dependent neuroprotector homeobox (ADNP), microtubule-associated proteins 1A/1 B light chain 3A (MAPILC3A), and matrix metalloproteinase 9 (MMP-9). SAC also attenuated inflammatory cell infiltration, axonal demyelination, and axonal loss in lumbar spinal cord in experimental autoimmune encephalomyelitis (EAE) group.

Activates Nrf-2

Shi H, Jing X, et al. 2015. J Neurochem. 133(2):298-308.

S-allyl cysteine (SAC) treatment resulted in an increase in nuclear factor erythroid-2-related factor 2 (Nrf2) protein levels and subsequent activation of antioxidant response element pathway genes in primary cultured neurons and models. Exposure of primary neurons to SAC provided protection against oxygen and glucose deprivation-induced oxidative insults. In wild-type ($Nrf2^{+/+}$) models, systemic administration of SAC attenuated middle cerebral artery occlusion-induced ischemic damage, a protective effect not observed in Nrf2 knockout ($Nrf2^{-/-}$) models. These findings provide the first evidence that activation of the Nrf2 antioxidant response by SAC is strongly associated with its neuroprotective effects against experimental stroke and suggest that targeting the Nrf2 pathway may provide therapeutic benefit for the treatment of stroke.

Franco-Eszastiga U, Santana-Martinez RA, et al. 2017.

S-allyl cysteine (SAC) were administered to models for 90 days activated transcription factor related to

Neurochem Res. 42(11):3041-51.

NF-E2 (Nrf2) factor in the hippocampus (25-200 mg/kg for 24 hours, i.g.) and striatum (100 mg/kg) and significantly decreased p65 levels in the frontal cortex (25-200 mg/kg). On the other hand, SAC increased glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) activities mainly in the hippocampus and striatum. Finally, the hippocampus showed a major level of 8-hydroxy-2-deoxyguanosine (8-OHdG) compared with the striatum and frontal cortex.

Brain, Neurotrophic, Anti-Aging and Anti-Depression Effects Review

Nishiyama N, Moriguchi T, et al. 1996. Shinyaku to Rinsho (New Drug Clin). 45:467-73.

Nishiyama et al. studied the effects of AGE and its compounds on neuron cell survival, memory loss and learning impairment using several aging model systems. In this review article, recent studies on the prevention of aging processes by AGE and its compounds were discussed.

Sumiyoshi H. 1999. Food Style 21. 3:36-40.

The effects of AGE on brain function were reviewed. AGE and one of its main compounds *S*-allylcysteine (SAC) have increased the survival rate of neuron cells and had a positive effect on establishing a new network between neuronal cells through improved branching. Structure-activity relationship studies determined the *S*-allyl group to be the most important moiety of the chemical structure. Physical and/or mental stress may cause a reduction in immunity. However, AGE is able to reduce stress and enhance natural killer (NK) activity. Also, inhibition of serotonin receptor 5-HT1B/1D binding by AGE may enhance brain function via improved immune system response. Memory and learning improvement is also observed with AGE.

Sumiyoshi H. 1999. Ch. 4. In: Food Development for Aging Prevention, pp. 170-7.

The effect of AGE and the prevention of aging were reviewed using recent brain and cardiovascular research. AGE and *S*-allylcysteine (SAC) have increased neuronal cell survival and have positive effects on establishing new networks between neuron cells through improved branching. Memory loss and learning were also improved by AGE using a model system. Several clinical studies demonstrated that AGE shows cholesterol binding effects, inhibits low-density lipoprotein (LDL) oxidation and protects endothelial cells from oxidized LDL-induced cell injury. All indicate that AGE may be useful for the prevention of aging processes.

Ray B, Chauhan NB, et al. 2011. Curr Med Chem. 18(22):3306-13.

The effects of AGE and one of its active ingredients *S*-allyl cysteine (SAC) in restricting several pathological cascades related to the synaptic degeneration and neuroinflammatory pathways associated with Alzheimer's disease (AD). Thus, based on the reported positive preliminary results reviewed herein, further research is required to develop the full potential of AGE and/or SAC into an effective preventive strategy for AD.

Qu Z, Mossine VV, et al. 2016. Neuromolecular Med. 18(3):474-82.

By utilizing quantitative proteomic approaches, AGE and two of its major consistuents, *S*-all yl-L-cysteine and N (α)-(1-deoxy-D-fructos-1-yl)-L-arginine, can attenuate neuroinflammatory responses in microglial cells through modulation of nuclear factor erythroid-2-related factor 2 (Nrf2)-mediated signaling as well as other oxidative stress-related pathways. These experimental data provide information for the molecular targets of AGE and its constituents to mitigate neurodegeneration and neuroinflammation and show a promising potential of these compounds.

Song H, Cui J, et al. 2020. Exp Ther Med. 19(2):1554-9.

AGE and sulfur-containing compounds, including *S*-allylcysteine (SAC) are well documented botanical active components of garlic. Studies have demonstrated both AGE and SAC can exert neuroprotective effects against neuroinflammation and neurodegeneration. Another bioactive component in AGE is N-α-(1-deoxy-D-fructos-1-yl)-L-arginine (FruArg). This review aims to provide an undated overview of the neuroprotective perspectives of these active garlic components (AGE, SAC and FruArg).

Sripanidkulchai B. 2020. Exp Ther Med. 19(2):1560-4.

AGE has been widely used to prevent chronic diseases, such as cancer and cardiovascular disease due to its high antioxidant activity. This review aimed to summarize the information related to the effects of AGE on learning memory in order to obtain a better understanding of its mechanisms of action and present an overview on the pathogenesis of Alzheimer's diease (AD), and summarizes the main ingredients and neuroprotective effects of AGE against cognitive and learning memory deficits.

Kosuge Y.2020. Exp Ther Med. 19(2):1565-9.

This review provides a current overiew of the protective actions of *S*-allyl-L-cysteine (SAC) and the SAC-related compounds, *S*-ethyl-L-cysteine (SEC) and *S*-propyl-L-cysteine (SPC), in neurodegenerative disease and discusses the promise of SAC as a prototype for developing novel therapeutic drugs for neurological diseases.

Other Pharmacological Effects

Anti-Bacterial Properties

Iimuro M, Kawamori T, et al. 2000. 6th Annual Meeting of Japanese Society for Helicobacter Research.

The effect of AGE on *Helicobacter pylori* (*H. pylori*)-induced gastritis was determined using a model system. Oral administration on *H. pylori* caused infection, dropsical swelling and reddening of the mucosa. AGE intake of 4% for 6 weeks reduced the reddening and thickening of the mucosa, suggesting AGE may reduce *H. pylori*-induced gastritis.

Anti-Fungal Properties

Matsuura H, Ushiroguchi T, et al. 1988. Chem Pharm Bull. 36(9):3659-63.

Matsuura et al. found that a constituent in AGE, furostanol glycoside, demonstrated anti-fungal effects by inhibiting the growth of *Candida albicans* in test tubes.

Abdullah TH, Kirkpatrick DV, et al. 1989. Deutsche Zeitschrift fuer Onkologie. 21:52-3.

AGE was found to improve candidiasis in AIDS patients.

Tadi PP, Teel RW, et al. 1990. Int Clin Nutr Rev. 10:423-9.

AGE hastened the clearance of *Candida albicans* from the circulation of models systemically infected with this organism. AGE reduced the number of these organisms growing in the kidneys where *Candida albicans* typically colonizes.

Improved Intestinal Conditions

Yokoyama K, Nagawa M, et al. 1990. Oyo Yakuri (Appl Pharmacol). 40(1):27-37.

AGE preparations were shown to be effective at improving gastrointestinal motor disorders induced by stress, such as delayed emptying of the stomach and intestine.

Gwilt P, Lear CL, et al. 1994. Cancer Epidemiol Biomarkers Prev. 3(2):155-60.

In a clinical study by Gwilt et al., AGE was found to enhance gastrointestinal motility.

Matsuura H. 1997. Ch. 7. In: Nutraceuticals: Desingers Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 55-69.

AGE was shown to enhance the growth of the friendly intestinal bacteria *Lactobacillus acidophilus* (*L. acidophilus*) and *Bifidobacterium bifidum* (*B. bifidum*) while other forms of garlic inhibited the growth of *B. bifidum*. Both *L. acidodophilus* and *B. bifidum* produce acid and antibacterial factors and have demonstrated an ability to decrease the growth of pathogens and their production of toxic and cancer-causing compounds in the intestinal tract.

Kokai Tokyo Koho. 1993. Japanese Patent H1-252276.

The effects of AGE on the growth of the beneficial bacteria *L. acidophilus* and *B. bifidum* can be attributed in part to the F-4 protein fraction in AGE.

Tatara MR, Sliwa E, et al. 2008. Ann Agric Environ Med. 15(1):63-9.

Models were given AGE daily orally at dosage of 1 and 2 ml/kg body weight. Results showed that AGE improved final body weight, morphological properties of intestinal villi and non-specific defense mechanisms of the models, indicating that AGE induced beneficial effects on health status, performance and systemic development of the gastrointestinal tract.

Berginc K, Zakelj S, et al. 2009. Biol Pharm Bull. 32(4):694-9.

AGE induced increase of the absolute value of the transepithelial potential difference and of the short-circuit current in both permeability models tested (jejunum, Caco-2 cell monolayers) without affecting transepithelial electrical resistance. It also caused a significant increase of the P-glycoprotein and multidrug resistance associated protein 2 mediated effluxes through jejunum of marker substrates Rhodamine 123 and 2,4-dinitrophenyl-*S*-glutathione increased significantly. The altered activity of transport proteins could significantly change the pharmacokinetic properties of conventional medicines taken with AGE.

Ried K, Travica N, et al. 2018. Front Nutr. 5:122.

Participants with uncontrolled hypertension completed a double-blind randomized placebo-controlled trial of 12 weeks. The group who consumed 1.2 g of Kyolic AGE daily was shown to have improved gut microbiota, evident by higher microbial richness and diversity with a marked increase in *Lactobacillus* and *Clostridia* species.

Protection from Heavy Metals

Wong SJ, Zhu DAH. The Effectiveness of S.G.P. on Dental Patients with Mercury Restorations-A Pilot Study.

In this unpublished study, Wong et al. found that 60 days administration of AGE led to increased excretion of mercury as measured by hair analyses. The average content of mercury excreted in hair was 1.565 parts per million (ppm). This pilot study suggests that AGE also has an *in vivo* chelation effect.

Lau BHS. 1989. Int Clin Nutr Rev. 9:27-31.

AGE has been shown to protect red blood cells (RBC) from oxidative damage caused by heavy metals. Various heavy metals (copper, mercury, aluminum and lead) were added to two sets of blood samples and AGE was added to one set. The heavy metals ruptured the RBC in each of the samples except for the cells to which AGE had been added. This experiment demonstrated a protective effect of RBC from AGE.

Protection from Insecticides

Ramadan G, El-Beih NM, et al. 2017. Environ Toxicol. 32(3):789-98.

Malathion and carbaryl are the most widely used organophosphate and carbamate insecticides, respectively, especially in developing countries. In toxicity induced by malathion and/or carbaryl in models, AGE containing 0.1% *S*-allylcysteine (SAC) (200 mg/kg body weight) for four consecutive weeks. AGE completely modulated most adverse effects induced by malathion and/or carbaryl in models including the normcytic normochromic anemia, immunosuppression, and the delay in the skin-burning healing process through normalizing the count of blood cells (erythrocytes, leucocytes and platelets), hemoglobin content, hematocrit value, blood glucose-6-phosphodehydrogenase activity, weights and cellularity of lymphoid organs, serum γ-globulin concentration, and the delayed type of hypersensitivity response to the control values, and accelerating the inflammatory and proliferative phases of burn-healing. AGE also completely modulated the decrease in serum reduced glutathione (GSH) concentration and the increase in clotting time in malathion alone and carbaryl alone treated models. Moreover, AGE induced a significant increase ($P < 0.001$) in serum GSH concentration (above the normal value) and accelerating burn-healing process in healthy models.

Ameliorated Effects of Titanium Dioxide

Abu Zeid EH, Alam RT, et al. 2017. Andrologia. 49(5).

Titanium dioxide (TiO_2)-exposed models significantly down-regulated androgen receptor (AR) mRNA expression and weak signal of AR immune labeling. Epithelium cell lining of seminal vesicles showed focal areas of necrosis and fibrous tissue with the prominent fibrous stroma of the atrophied glands. However, AGE supplementation ameliorated the deleterious effects of TiO_2 intoxication through protecting the tissues from oxidative stress caused by TiO_2.

Moustafa GG, Hussein MM. 2016. Biomed Pharmacother. 84:687-97.

The administration of AGE to titanium dioxide (TiO_2)-intoxicated models alleviated the hepatic inflammation and pulmonary fibrotic responses induced by TiO_2.

Nutritional Support for Genital and Oral Herpes

Tseui J. June 4, 1987. International Publication Number WO97/03203. International Patent Classification: A61K 35/78.

A patent for AGE was completed for oral and genital herpes. AGE can be used orally or topically, and delays and minimizing the symptoms of the virus, as well as increasing the period of time between recurrences of viral shedding.

Abdullah TH, Kirkpatrick DV, et al. 1989. Deutsche Zeitschrift fuer Onkologie. 21:52-3.

AIDS patients given a 6 week course of AGE noted interruptions of recurrent cycles of genital herpes.

Effects of AGE on Sugar Metabolism

Nagai K, Nagakawa S, et al. 1975. Yakuri to Chiryo (Jpn J Pharmacol Ther). 3(1):45-53.

The group given Kyoleopin® (KLE) showed good control of their blood glucose level, especially in the group that received 8g/kg of glucose load.

Kasuga S, Itakura Y, et al. 1999. Nippon Yakurigaku Zasshi (Folia Pharmacol Jpn). 114:191-7.

Pretreatment with AGE (5 and 10 ml/kg, p.o.) significantly prevented adrenal hypertrophy, hyperglycemia and elevation of corticosterone, without altering insulin level, exposed to 2 days (16 hours/day) of immobilization stress. From these results, AGE was suggested to prevent stress-induced hyperglycemia, which is the risk of suffering from diabetes mellitus and its progression.

Blood Building Effects of AGE

Nagai K, Nakagawa S, et al. 1975.

Blood (1/200 volume of body weight) was withdrawn from models to determine the blood building effects

Yakuri to Chiryo (Jpn J Pharmacol Ther). 3(4):659-66.

of Kyoleopin® (KLE). The group taking KLE, when compared to the control, showed a remarkably slower rate of decrease in the number of red blood cells, hematocrit, hemoglobin and body weight loss. The appearance of reticulocytes was also decreased in the KLE group. KLE also showed increased hematopoietic or red blood cell production activity of the bone marrow.

Hasegawa T, Kikuchi N, et al. 1984. Shinryo to Shinyaku (Treat New Med). 21(10):2021-35.

In a clinical study of 132 subjects suffering from indefinite medical complaints, a noteworthy improvement in anemia was found.

Improved Male Reproductive Function

Kasuga S, Uda N, et al. 2001. J Nutr. 131(3 Suppl):1080S-4S.

The pharmacological activities of four garlic preparations including raw garlic juice (RGJ), heated garlic juice (HGJ), dehydrated garlic powder (DGP) and AGE were investigated using testicular hypogonadism (hypospermatogenesis and impotence) induced by warm water treatment. RGJ effectively recovered testicular functions, DGP restored recovery of spermatogenesis and AGE was also effective. HGJ had no effect on impotence.

Morihara N, Ushijima M, et al. 2001. 121st Annual Meeting of the Pharmaceutical Society of Japan. Sapporo, Japan. Mar 28-30. Abst #28(PB)II-006.

The effects of tonic herbal medicines on male reproductive dysfunction induced by hyperthermic treatment were studied. AGE demonstrated spermatogenesis promotion while raw and boiled garlic extract did not. When observing the mechanism, Morihara et al. found that AGE acted differently from testosterone by improving the peripheral blood circulation which may play an important role in spermatogenesis promotion.

Nishimatsu H, Kitamura T, et al. 2014. Aging Male. 17(2):112-6.

Leopin-Royal® (LER), a preparation containing AGE, ginseng, Oriental bezoar, velvet antler, cuscuta seed and epimedium herb or Kampo (mainly kamishoyan) as a control, were administered to 49 male patients with mild or more pronounced symptoms of aging in a randomized clinical trial for 6 months. A decrease in the slope of the LER group was greater than that of the Kampo group. There was a significant difference between the groups and the group and month interaction (G × M) ($p<0.05$). The International Index of Erectile Function with 5 questions (IIEF-5) score increased in the LER group ($p = 0.02$ with regard to G × M), indicating that LER is superior to Kampo on the rate of improvement of symptoms of aging, including erectile dysfunction, in males.

Reduced Gingival Inflammation and Gingival Bleeding

Zini A, Mann J, et al. J Clin Dent. 2018. 29(2):52-6.

In a randomized, controlled, examiner- and participant-blind, two treatment parallel group study participants were given 4 capsules of Kyolic Reserve (2.4 g AGE) or placebo daily for 4 months. There was a stastistical significant decrease in gingival inflammation and gingival bleeding in the AGE group compared to placebo ($p < 0.001$) which suggests daily consumption of AGE may benefit oral health.

Overview of Various Effects

Yeh YY. 1996. Shinyaku to Rinsho (New Drug Clin). 45(3):441-50.

Various pharmacological effects of AGE were presented. These effects included its ability to stimulate phagocytic function of macrophages, proliferate lymphocytes and remove *Candida albicans* from the blood circulation of models, its antioxidative effects, its ability to prolong life and improve memory retention of senescence-accelerated models and its potential as a functional food, food supplement and means of curbing health care costs.

Hatono S. 1996. Shinyaku to Rinsho (New Drug Clin). 45(3):474-81.

Since ancient times, garlic has been used for the treatment and prevention of disease all over the world. Generally, sulfur compounds are the main constituents found in garlic. However, cooking and/or processing through boiling, cutting and/or aging will change the content and species of these compounds. Previous reports indicate that different cooking and/or processing methods cause different pharmacological activities and safety. Among the different garlic products, AGE has been studied based on its various pharmacological activities, such as anti-tumor and anti-fungal effects, antioxidant properties and preventing cardiovascular diseases.

Sumiyoshi H. 1997. Nippon Yakurigaku Zasshi (Folia Pharmacol Jpn). 110:93-7.

Sumiyoshi H. 1997. 25th Pharmacological Activity Symposium. Tokyo, Japan. Oct 30-31.

Pharmacological findings suggest garlic has more preventive rather than therapeutic benefits. The anti-carcinogenic, cardioprotective and immune-stimulating effects of AGE components such as *S*-allyl cysteine (SAC) have been demonstrated.

Kyo E. 2003. Igaku no Ayumi (Progress in Medicine). 204:74-9.

Kyo E. 2003. J Jpn Mibyou System Assoc. 9:51-4.

Garlic has been used as folk medicine for more than 5000 years. Its variety of efficacies has been reported and more than 3000 scientific papers have been published on its chemistry, safety and biological activities. There are many different kinds of garlic products on the market, including AGE. In this review article, the various pharmacological effects of AGE, including its anti-tumor effects, were discussed.

SAFETY OF AGED GARLIC EXTRACT

General Toxicity

Nakagawa S, Masamoto K, et al. 1980. J Toxicol Sci. 5(1):91-112.

No growth retardation, stomach injuries, changes in red blood cell count or morphological abnormalities were observed in models given 5 ml/kg AGE for 3-21 days. On the other hand, consumption of 5 ml/kg raw garlic juice led to a decrease in total serum protein and albumin, acute inflammation of the stomach and stomach ulcers, decreases in red blood cells and hemoglobin, and increases in serum bilirubin. Nakagawa et al. concluded that when garlic is aged, the toxicity is greatly reduced since none of the side effects observed in the raw garlic group were observed in the AGE group.

Kanezawa A, Nakagawa S, et al. 1984. Oyo Yakuri (Appl Pharmacol). 27:909-29.

The median lethal dose (LD_{50}) for Kyoleopin® (KLE) for oral and subcutaneous administration was over 30 ml/kg, the maximum amount physically possible to administer to models. One-time oral administration caused inhibition of spontaneous movements due to abdominal distention. Subcutaneous administration led to thickening of the skin and granulation where administered. However, no death due to KLE occurred during the 7-day observation period.

For long-term administration, adverse effects such as decreased red blood cells, hemoglobin and slight increase in spleen weight, were noted only in dosages 250 times the usual dosage. Even in such cases, no abnormal pictures of red blood cells were observed.

Hoshino T, Kashimoto N, et al. 2001. J Nutr. 131(3 Suppl):1109S-13S.

Enteric-coated garlic supplements have been designed to deliver garlic powder directly to the intestinal tract. Using the newly developed endoscopic compressed air delivery system. Hoshino et al. assessed the effects of garlic powders on the gastrointestinal mucosa. Raw garlic cloves, boiled garlic cloves and AGE, freeze-dried and pulverized into powder, were administered into the stomach and their effects on the mucosa were endoscopically determined 24 hours after administration. Fresh garlic powder caused severe damage including erosion and ulcer-like lesions. Boiled garlic powder caused a reddening of the mucosa, whereas AGE powder caused no undesirable effects. These results suggest that the safety should be seriously considered when choosing a garlic supplement.

Acute Toxicity

Nakagawa S, Masamoto K., et al. 1984. J Toxicol Sci. 9(1):57-60.

In an acute toxicity test, Nakagawa et al. estimated the median lethal dose (LD_{50}) of AGE to be over 30 ml/kg. This is the maximum amount that could be physically delivered.

Chronic Toxicity

Sumiyoshi H, Kanezawa A, et al. 1984. J Toxicol Sci. 9(1):61-75.

No toxic symptoms in the tissues, organs or cells given AGE were observed even at dosages of 2000 mg/kg 5 times per week for 6 months.

Mutagenicity

Yoshida S, Hirao Y, et al. 1984. J Toxicol Sci. 9:77-86.

Various tests showed no evidence of mutagenicity of AGE. AGE also did not affect the incidence of micronucleated cells and polychromatic cells, whereas raw garlic juice increased the incidence of such damaged cells. Though cells cultured in fresh garlic juice showed signs of growth inhibition and morphological changes, those cultured in AGE did not. No signs of cytotoxicity were observed, even at the highest concentration of AGE, only slight signs were seen.

Acute and Subacute Toxicity

Imada O. 1990. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. Aug 28-30, p. 47.

Imada concluded the following:

1) Raw garlic, allicin and diallyl disulfide (DADS) are toxic when large doses are taken.
2) When garlic is aged, its toxicity is greatly reduced and a commercially available AGE is almost without toxicity even when a very large dose is taken.
3) The toxicity of oil-soluble garlic constituents is higher than water-soluble constituents.

Nakagawa S, Sumiyoshi H, et al. 1984. Oyo Yakuri (Appl Pharmacol). 27(6):1133-50.

It was noted that oral median lethal dose (LD_{50}) for Leopin-5® (LE-5), was over 30 ml/kg. No toxic signs were seen within 7 days after administration. At 10 ml/kg, a slight decrease in food intake was noted

though there was no change in weight and no toxic signs were seen for 3 consecutive months. At excessively high dosage, red blood cell count and hemoglobin value decreased slightly while spleen and liver weights increased a little. There were no toxic signs observed in any of the tissues or organs examined.

Safety During Pregnancy

Masamoto K, Sumioka I, et al. 1994. In House Data from Wakunaga Pharmaceutical Co., Ltd.

The safety of AGE has been confirmed in preclinical studies at each trimester of pregnancy. AGE Liquid has been successfully recommended for more than 50 years as an over-the-counter medicine for pregnant women in Japan.

Drug Interactions with AGE

—Coumadin

Rozenfeld V, Sisca T, et al. 1999. Eighteenth Annual Eastern States Conference for Pharmacy Residents and Preceptors. Baltimore, MD. Apr 21-24. 33, Abst #42.

Rozenfeld V, Sisca TS, et al. 2000. Am Society of Health-System Pharmacists (ASHP). Las Vegas, NV. Dec 3-7. Abst #35P.

Safety intake of AGE with Coumadin® has become a question since both show blood-thinning properties. A double-blind, placebo-controlled clinical trial suggests that there is no toxic synergism between these substances and that AGE is safe in conjunction with Coumadin®.

Amagase H, Niihara Y, et al. 2004. International Congress on Natural Products Research. Jul 31-Aug 4.

No evidence of increased hemorrhage was observed in a clinical study with deep vein thrombosis (DVT) patients on oral anticoagulation (Coumadin®) therapy who were administered 5 ml of AGE twice a day for 12 weeks. AGE is relatively safe and poses no serious hemorrhage risk for patients on Coumadin® therapy with close monitoring.

Macan H, Uykimpang R, et al. 2006. J Nutr. 136(3 Suppl):793S-5S.

AGE was admininstered at a dose of 5 ml twice a day for 12 weeks in a double-blind, randomized, placebo-controlled pilot study. There was no evidence of increased hemorrhage in either the placebo or the AGE group.

—Statins and Aspirin

Budoff M, Takasu J, et al. 2004. Prev Med. 39(5):985-91.

A double-blind, randomized, placebo-controlled clinical study using AGE on cardiovascular patients has shown no contraindications with statin and aspirin.

—Acetaminophen

Gwilt P, Lear CL, et al. 1994. Cancer Epidemiol Biomarkers Prev. 3(2):155-60.

Except for a slight increase in sulfate conjugation, Gwilt et al. found that AGE did not affect the metabolism or efficacy of acetaminophen.

—Saquinavir

Borek C. 2002. Clin Infect Diseases. 35(3):343.

Dehydrated garlic powder product has been reported to have a drug interaction, especially AIDS treatment drug, such as Saquinavir, that is influenced by cytochrome P450 metabolism enzymes. AGE may not be necessary to have such negative interaction with medicines since it has a special preparation method to eliminate odorous oil-soluble sulfur compounds by aging extraction process. This note has clearly pointed out this difference and arguments with original authors of the paper.

—Saquinavir and Darunavir

Berginc K, Milisav I, et al. 2010. Drug Metab Pharmacokinet. 25(6):521-30.

AGE significantly inhibited saquinavir efflux from model hepatocytes, while the efflux of darunavir significantly increased. Phytochemicals, inducing saquinavir and darunavir distribution changes were most probably flavonoids and lipophilic organosulfur compounds, respectively. AGE also inhibited CYP3A4 metabolism of both drugs and modulated hepatic distribution of the corresponding saquinavir's and darunavir's metabolites. The competition between saquinavir and garlic constituent(s) for the same binding site on the efflux transporter and the positive-cooperative effect between darunavir and garlic phytochemical(s), which bind to separate binding places on transporter, are the most probable

mechanisms to explain plasma profile changes, which could occur *in vivo* during concomitant consumption of antiretrovirals and garlic supplements.

Berginc K, Trontelj J, et al. 2010. Drug Metab Pharmacokinet. 25(3):307-13.

The presence of AGE in an incubation medium with HIV protease inhibitors saquinavir and darunavir caused a significant inhibition of saquinavir efflux from human hepatocellular carcinoma HepG2 cells and precision cut liver slices, while the activity of darunavir efflux transporters in both liver models significantly increased. Due to the opposite *in vitro* interactions observed between AGE and HIV protease inhibitors, saquinavir and darunavir most probably bind to different binding sites on one or both efflux transporters.

Berginc K, Trdan T, et al. 2010. Biopharm Drug Dispos. 31(8-9):495-505.

Saquinavir and darunavir efflux from enterocytes into gastrointestinal lumen (*in vitro* permeability through model jejunum) significantly increased in the presence of AGE, whereas their CYP3A4 metabolism was inhibited. Therefore, the fractions of tested anti-HIV drugs absorbed could decrease significantly during self-medication with garlic supplements. Due to distinct saquinavir and darunavir preferences for binding sites on efflux transporters, the presence of garlic phytochemicals, capable of influencing intestinal transporter-enzyme interplay, might lead to pharmacokinetic interactions observed in clinical studies and case reports with anti-HIV drugs.

—Others

Berginc K, Zakelj S, et al. 2010. Eur J Nutr. 49(6):373-84.

In a model small intestine and human epithelial colorectal adenocarcinoma cells (Caco-2) monolayers mounted side-by-side diffusion chambers, hydrophilic sulfur compounds from AGE increased P-glycoprotein (Pgp) mediated Rhodamine 123 (Rho123) efflux, whereas lipophilic ones increased Pgp efflux through model ileum but not through Caco-2 cell monolayers. Increased activities of secretory (Pgp, multidrug-resistance associated protein 2) and absorptive (monocarboxylate transporter 1, organic anion transporting polypeptide) transporters involved in drug absorption were observed in model small intestine and Caco-2 monolayers in the presence of AGE.

Berginc K, Kristl A. 2011. Curr Drug Metab. 14(1):90-101.

This paper summarizes the mechanisms responsible for first-pass intestinal pharmacokinetic interactions by investigating the intestinal permeability of some cardiovascular, antiviral drugs, their transport with hepatic transporters and cytochrome P450 3A4 (CYP3A4) metabolism. Garlic phytochemicals from AGE modified the activities of secretory and absorptive transporters in both intestine and liver and competitively inhibited CYP3A4 enzyme.

Dosages in Studies

Kandil OM, Abdullah TH, et al. 1987. Fed Proc. 46(3):441. Abst #723.

Abdullah TH, Kirkpatrick DV, et al. 1989. Deutsche Zeitschrift fuer Onkologie. 21:52-3.

Gwilt P, Lear CL, et al. 1994. Cancer Epidemiol Biomarkers Prev. 3(2):155-60.

Steiner M, Khan AH, et al. 1996. Am J Clin Nutr. 64(6):866-70.

Yeh YY, Lin RI, et al. 1997. In: Food Factors for Cancer Prevention. Ohgashi H (ed). Springer-Verlag, Tokyo, pp. 226-30.

Dimitrov NV, Bennink MR. 1997. Ch. 21. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 199-202.

Steiner M, Li W. 2001. J Nutr. 131(3 Suppl):980S-4S.

The majority of the original clinical studies conducted utilized from 2-4 ml of AGE liquid, roughly equivalent to 1,200 mg of the powder, thus establishing the suggested daily intake of 1,200 mg. In clinical studies, as little as 1,800 mg of AGE powder has shown immunostimulatory activity, enhancing natural killer (NK) cell activity in normal subjects (Kandil et al. 1987), whereas as much as 10,000 mg has been safely taken by AIDS patients and has shown similar enhancement of these cells over a 6-week period (Abdullah et al. 1989). In 2 double-blind clinical studies, 7,200 mg of AGE powder has shown to effectively reduce cholesterol, blood pressure and platelet aggregation having been safely taken for 5-6 months (Steiner et al. 1996 and Yeh et al. 1997). Another double-blind, randomized, placebo-controlled study (Steiner et al. 2001) showed lower dosages (2.4 g and 4.8 g) of AGE are significantly effective at thinning the blood. A clinical study (Gwilt et al. 1994) utilized 10 ml per day of AGE liquid for 3 months and found increased sulfate conjugation of the analgesic acetaminophen. Even at this high dosage (more than twice the suggested daily dosage), no body odor was noted from subjects. Another study (Dimitrov et al. 1997) also utilized 10 ml/day showing a decrease in the levels of serum prostaglandins and it was tolerated well by all participants.

Side Effects

Lau BHS, Lam F, et al. 1987. Nutr Res. 7:139-49.

No severe side effects were noted in the more than 40 clinical studies using AGE confirming the safety of such preparations. The preparations were generally well-tolerated, even at high dosages. Minor side effects, noted in only a few clinical studies, were few in number and less than 10% of the subjects. Main complaints included stomach discomfort, nausea, flatulence, diarrhea and headaches, which suggests a psychological factor possibly plays a role in side effects.

S-ALLYL CYSTEINE (SAC): A KEY COMPOUND IN AGED GARLIC EXTRACT

Amagase H, Matsuura H, et al. 2000. Ch. 6. In: Phytochemicals and Phytopharmaceuticals. AOCS Press. Champaign, IL, pp. 62-78.

Garlic Fluidextract. 2002. United States Pharmacopeia-National Formulary (USP25 NF20). United States Pharmacopeia Convention. Rockville, MD, p. 2553.

AGE contains numerous compounds that have demonstrated beneficial effects and a synergy of these compounds is likely responsible for the benefits of AGE. For the sake of quality control, AGE is standardized with *S*-allyl cysteine (SAC), a stable, effective and safe organosulfur compound derived from garlic, which significantly and naturally increases during the aging process. AGE preparations are standardized to contain no less than 0.05% SAC by dry weight. The following studies suggest that SAC can provide protection against oxidation, free radicals, pollution, cancer and cardiovascular diseases. The bioavailability of SAC has also been confirmed in several models.

Cardiovascular Effects of SAC

Cholesterol Lowering Effect

Qureshi N Lin RIS, et al. 1990. First World Congress on the Health Significance of Garlic and Garlic Constiutents. Washington, D.C. Aug 28-30, p. 17.

Qureshi AA Lin RIS, et al. 1990. First World Congress on the Health Significance of Garlic and Garlic Constiutents. Washington, D.C. Aug 28-30, p. 16.

SAC was found to lower serum cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides in both normolipidemic and hypercholesterolemic models. Cholesterol was lowered by the inhibition of key enzymes in cholesterol synthesis, β-hydroxy-β-methylglutaryl CoA synthetase and reductase, in the liver. Key enzymes of lipogenesis acetyl CoA carboxylase and fatty acid synthetase were also significantly inhibited by SAC.

Among garlic preparations tested, maximum inhibition of cholesterol-producing enzyme activities was observed in this order: **Kyolic® AGE > SAC > commercial garlic oil < garlic powder**

Abuirmeileh N, Yu SG, et al. 1991. FASEB J. 5(6):A1756. Abst #8048.

SAC lowered total serum cholesterol and low-density lipoprotein (LDL) cholesterol in hypercholesterolemic models. Cholesterol was lowered by inhibiting the activity of the key enzymes in cholesterol synthesis, β-hydroxy-β-methylglutaryl CoA synthetase and reductase, in the liver.

Yeh YY, Yeh SM. 1994. Lipids. 29(3):189-93.

SAC inhibited the synthesis of cholesterol and fatty acids in cultured liver cells.

Liu L, Yeh Y. 1999. FASEB J. 13(4):A556. Abst #442.7.

Yeh YY, Liu L. 2001. J Nutr. 131(3 Supp):989S-93S.

Water-soluble organosulfur compounds, such as *S*-alk(en)yl cysteines (SAC, *S*-ethylcysteine, etc.) and γ-glutamyl-*S*-alk(en)yl cysteines (γ-glutamyl-*S*-allylcysteine, γ-glutamyl-*S*-methylcysteine, etc.), derived from garlic inhibited 20-60% of the cholesterol biosynthesis in primary cultured hepatocytes, apparently through metabolic alteration. Alliin, a major water-soluble organosulfur constituent in garlic cloves, had no inhibitory effect. Oil-soluble sulfur compounds including diallyl disulfide and diallyl trisulfide, also inhibited synthesis (10-15%), however, inhibition by these compounds was accompanied by the release of intracellular lactate dehydrogenase, indicating the reduction of cholesterol synthesis could be due to cytotoxicity. Based on half maximal inhibitory concentration (IC_{50}) and maximal inhibition, SAC was found to be one of the major garlic constituents responsible for non-toxic cholesterol reduction.

Lee Y, Yeh Y-Y. 2003. FASEB J. 17(4):A752. Abst #455.1.

Water-soluble organosulfur compounds in Kyolic: SAC, *S*-ethyl cysteine (SEC), *S*-propyl cysteine (SPC) and gamma glutamyl *S*-alk(en)yl cysteines, have shown maximal inhibition on cholesterol synthesis requires a concerted action of these various compounds in human hepatocellular carcinoma (HepG2) cells.

Malekpour-Dehkordi Z, Javadi E, et al. 2013. Phytother Res. 27(3):357-61.

ATP-binding cassette transporter A1 (ABCA1) is a key mediator of cholesterol efflux to apoA-I in lipid loaded macrophages, which is the first step of reverse cholesterol transport *in vivo* and is critical in preventing atherosclerosis. Human monocyte THP-1 cells were differentiated to macrophage cells were then treated with different concentrations (10, 20 and 40 mM) of SAC for 24 hours. Results showed that SAC increased ABCA1 mRNA (1.82-, 2.07- and 2.23-fold) and protein (1.37-, 1.55- and 2.08-fold) expression in macrophage THP-1 cells compared with control (untreated cells).

Asdaq SM. 2015. Evid Based Complement Alternat Med. 328545.

In models treated with AGE and SAC, there was a significant decline in elevated levels of triglyceride, total cholesterol, alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), malondialdehyde, glutathione peroxidase enzyme activity, total glutathione and oxidized glutathione in serum and inclined superoxide dismutase, catalase, ferric reducing/antioxidant powder, and total sulfhydryl values in liver tissue with reduction in thiobarbituric acid reactive species.

Blood-Thinning Effect

Qureshi AA Lin RIS, et al. 1990. First World Congress on the Health Significance of Garlic and Garlic Constiutents. Washington, D.C. Aug 28-30, p. 16.

Abuirmeileh N, Yu SG, et al. 1991. FASEB J. 5(6):A1756. Abst #8048.

Yu SG, Qureshi N, et al. 1991. National Conference on Cholesterol and High Blood Pressure. Washington, D.C. Apr 8-10.

SAC lowered the levels of plasma thromboxane B_2 and factor 4 (blood clotting factors) in hypercholesterolemic models up to 30%. SAC also decreased platelet aggregation induced by the potent clotting agents, collagen and adenosine diphosphate.

Hypotensive/Blood Pressure Lowering Effect

Cruz C, Correa-Rotter R, et al. 2007. Am J Physiol Renal Physiol. 293(5):F1691-8.

SAC was given to nephrectromized models (200 mg/kg, i.p.) every other day for 30 days. SAC reduced hypertension, suggesting that the antihypertensive effect of SAC is associated with its antioxidant properties and that it may be used to ameliorate hypertension.

Shouk R, Abdou A, et al. 2014. Nutr Res. 34(2):106-15.

This review discusses the molecular, biochemical and cellular rationale underlying the antihypertensive properties of garlic and its bioactive constituents, including *S*-allylcysteine (SAC). SAC has been shown in studies to modulate various parameters implicated in the pathogenesis of hypertension, which include oxidative stress, nitric oxide bioavailability, hydrogen sulfide production, angiotensin converting enzyme activity, expression of nuclear factor κB and the proliferation of vascular smooth muscle cells. This suggests that garlic and garlic derived bioactives have significant medicinal properties with the potential for ameliorating hypertension and associated morbidity.

Other Cardioprotective Effects

Chuah SC, Moore PK, et al. 2007. Am J Physiol Heart Circ Physiol. 293(5):H2693-701.

SAC significantly lowered mortality and reduced infarct size of acute myocardial infarction (AMI) in models. SAC also increased left ventricular cystathionine-γ-lyase (CSE) activity, which is the enzyme responsible for hydrogen sulfide (H_2S) production in the heart ($P<0.01$), thus, also had higher plasma H_2S concentration compared with controls and the SAC + propagylglycine (PAG)-treated group. Protein expression studies revealed that SAC upregulated CSE expression (1.1-fold of control; $P<0.005$). This study provides novel evidence that SAC is protective in myocardial infarction via a H_2S-related pathway.

Padmanabhan M, Rajadurai M, et al. 2008. Basic Clin Pharmacol Toxicol. 103(6):507-13.

Models were pre-treated with SAC (50, 100 and 150 mg/kg) daily for a period of 45 days then subcutaneously injected with isoproterenol (150 mg/kg) at an interval of 24 hours for 2 days. Pre-treatment with SAC exhibited significant ($P<0.05$) effect and positively altered the biochemical parameters (activities of serum creatine kinase-MB [muscle and brain isozyme]), calcium-dependent adenosine triphosphatase and magnesium-dependent adenosine triphosphatase in the heart, serum levels of iron and uric acid, levels of plasma iron binding capacity, plasma total protein, plasma albumin/globulin ratio and activity of sodium potassium-dependent adenosine triphosphatase in the heart, levels of glycoproteins in the serum and heart). From this study, SAC showed a protective role in isoproterenol-induced myocardial infarction in models, which may be due to free radical scavenging, antioxidant and membrane stabilizing properties.

Malekpour-Dehkordi Z, Javadi E, et al. 2013. Phytother Res. 27(3):357-61.

The human monocyte THP-1 cells were differentiated to macrophage cells in the presence of phorbol 12-myristrate13-acetate (PMA) then treated with different concentrations of SAC for 24 hours. Results showed that SAC increased the adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1) messenger RNA (mRNA) (1.82-, 2.07- and 2.23-fold) and protein (1.37-, 1.55- and 2.08-fold) expression in macrophage THP-1 cells compared with control (untreated cells). These results suggested that SAC can increase ABCA1 expression in macrophages and may be beneficial in promoting reverse cholesterol efflux.

Avula PR, Asdaq SM, et al. 2014. Indian J Pharmacol. 46(1):94-9.

Models were administered AGE at two different doses of 2 ml/kg or 5 ml/kg orally, whereas SAC was administered either at a dose of 13.1 mg/kg or 32.76 mg/kg, given alone or in combination with atenolol, every alternate day for 3 weeks. Two doses of isoproterenol were administered to models at the end of treatment. AGE and SAC administration caused a decrease in serum lactate dehydrogenase (LDH) and creatinine kinase-MB (CK-MB) activities and an elevation of LDH and CK-MB activities in heart tissue homogenate (HTH). Atenolol alone or in combination with AGE and SAC demonstrated similar changes

in biomarker activities. AGE showed dose-dependent cardioprotection while SAC with atenolol combated more effectively the myocardial dysfunction during isoproterenol induced cardiotoxicity in models.

Inhibition of Vascular Smooth-Muscle Cell and Umbilical Endothelial Cell Proliferation

Yamasaki T, Lin L, et al. 1994. Phytother Res. 8:408-12.

Yamasaki et al. found that SAC can protect cells that line the blood vessels of the lungs from oxidant injury. In this *in vitro* test, SAC protected bovine pulmonary endothelial cells from hydrogen peroxide (H_2O_2)-induced oxidant injury. Pretreatment of cells overnight with SAC (4 mg/ml) significantly reversed the loss of cell viability, inhibited lactate dehydrogenase (LDH) release and lipid peroxidation induced by H_2O_2. Based on these results, it was suggested that that AGE and SAC may be effective in hampering the aging process and for the prevention of atherosclerosis.

Lee ES, Steiner M, et al. 1994. Biochim Biophys Acta. 1221(1):73-7.

SAC was found to inhibit vascular smooth-muscle cell (SMC) and umbilical endothelial cell proliferation. SMC proliferation constitutes an essential aspect in the development of atherosclerosis and of restinosis or narrowing/constriction of blood vessels subjected to angioplasty.

Sickle Cell Anemia

Ohnishi ST, Ohnishi T. 2001. J Nutr. 131(3 Suppl):1085S-92S.

Sickle cell anemia is a genetic disease caused by abnormal hemoglobin. By exposing sickle red blood cells to deoxy-oxy cycling *in vitro*, dense red cells were formed. Dense cells can be found in patients and they may cause blood vessel occlusion. Using this method, SAC was shown to inhibit the formation of dense cells by 30% at 1 mg/ml concentration *in vitro*.

Takasu J, Uykimpang R, et al. 2002. BMC Blood Disorders. 2(1):3.

The potential role of AGE as an antioxidant for sickle red blood cells (RBC) was examined. Unanimously, the patient's count of Heinz bodies decreased from 58.9% to 29.8% during the 4 weeks of the study. This data suggests the significant antioxidant activity of AGE on sickle cell anemia and may represent a potential therapy to combat complications of the disease.

Diabetes

Ahmad MS, Ahmed N. 2006. J Nutr. 136(3 Suppl):796S-9S.

Ahmad MS, Pischetsrieder M, et al. 2007. Eur J Pharmacol. 562(1-3):32-8.

Elosta A, Slevin A, et al. 2007. 9th International Symposium on Maillard Reaction. The Maillard Reaction. Munich, Germany. Sep 1-5, p. 161. Poster # BM7.

SAC, the key component in AGE, was shown to inhibit advanced glycation endproduct (AGEP) formation *in vitro*, which can help to prevent diabetic complications.

Saravanan G, Ponmurugan P. 2010. Plant Food Hum Nutr. 65(4):374-8.

The possible protective effects of SAC on the antioxidant defense system of pancreas in streptozotocin (STZ)-induced diabetes in models were evaluated. The levels of glucose, thiobarbituric acid reactive substances (TBARS), and enzymatic antioxidants reverted back to near control levels after treatment with SAC. These findings suggest that SAC treatment exerts a therapeutic protective nature in diabetes by decreasing oxidative stress.

Saravanan G, Ponmurugan P, et al. 2010. Phytomedicine. 17(14):1086-9.

SAC was administered orally for 45 days to normal and streptozotocin-induced (STZ) diabetic models. STZ-induced diabetic models showed significant increase in blood glucose and glycoprotein components such as hexose, hexosamine, ructose and sialic acid in plasma, liver and kidneys of diabetic models. SAC administration normalized all the above-mentioned biochemical parameters. This study indicates that SAC possesses a significantly beneficial effect on the glycoprotein moiety in addition to its antidiabetic effect.

Saravanan G, Ponmurugan P. 2011. Exp Toxicol Pathol. 64(6):639-44.

Oral administration of SAC at a dose of 150 mg/kg bodyweight per day to streptozotocin (STZ)-induced diabetic models for a period of 45 days resulted in a significant reduction in fasting blood glucose, cholesterol (TC), triglycerides (TG), free fatty acids, phospholipids, low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and elevation of high-density lipoprotein cholesterol (HDL-C) in comparison with diabetic control group. SAC administration to diabetic models also decreased the concentrations of fatty acids, namely palmitic, stearic (16:1) and oleic acid (18:1), whereas linolenic (18:3) and arachidonic acid (20:4) were elevated. The results indicate that SAC showed an antihyperlipidemic effect in addition to its antidiabetic effect in experimental diabetes.

Saravanan G, Ponmurugan P. 2011. Chem Biol Interact. 189(1-2):100-6.	SAC was administered orally for 45 days to control and streptozotocin (STZ)-induced diabetic models. SAC administration to models showed a decrease in plasma glucose, thiobarbituric acid reactive substances (TBARS), hydroperoxide and oxidized glutathione (GSSG). In addition, the levels of plasma insulin, superoxide dismutase, catalase, glutathione peroxidase (GPx) and reduced glutathione (GSH) were increased in SAC-treated diabetic models, which these findings were supported by histological observations of the liver and kidney. This indicates that SAC possesses a significant favorable effect on antioxidant defense system in addition to its antidiabetic effect.
Saravanan G, Ponmurugan P. 2012. J Diabetes Complications. 26(4):280-5.	SAC was administered orally for 45 days to control and streptozotocin (STZ)-induced diabetic models. SAC administration to diabetic models showed a decrease in plasma glucose, thiobarbituric acid reactive substances (TBARS), hydroperoxide and glycated hemoglobin (HbA1C). In addition, the levels of plasma insulin, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), total protein, albumin, thyroid hormone and thyroid-stimulating hormone (TSH) were increased in SAC-treated diabetic models. From these findings, SAC might be acting through activation in the synthesis and/or secretion of circulating thyroid hormones which in turn stimulate the synthesis of insulin.
Saravanan G, Ponmurugan P, et al. 2013. J Trace Elem Med Biol. 27(2):143-7.	SAC was administered orally to control or streptozotocin (STZ)-induced diabetic models for 45 days. The levels of glucose, iron, ferritin, bilirubin and heart heme oxygenase activity (HO) in liver were increased significantly ($p<0.05$) whereas the levels of insulin, transferrin and δ-aminolevuliniacid dehydratase activity (δ-ALA-D) in tissues were decreased in diabetic models. Administration of SAC to diabetic models showed a decrease in blood glucose, iron, ferritin, bilirubin and HO. In addition, the levels of insulin, transferrin and δ-ALA-D activity in tissues were increased in SAC-treated diabetic models.
Nirala BK, Gohil NK. 2015. Eur Rev Med Pharmacol Sci. 19(11):2125-31.	In the presence of SAC sulfoxide (SACSO) was shown to exert an attenuating effect on 20 mM glucose derived advanced glycation end products (AGE)-human serum albumin (HSA) induced inflammation, by selectively inhibiting RAGE (triggers inflammatory pathways) while stimulating galectin-3 expression (sequesters AGE molecules and prevents AGE-RAGE engagement) *in vitro*. Also, nitric oxide synthase (NOS) activity was enhanced and expression of inflammatory marker soluble intercellular adhesion molecule-1 (sICAM-1) was reduced.
Baluchnejadmojarad T, Kiasalari Z, et al. 2016. Eur J Pharmacol. 794:69-76.	Streptozotocin (STZ)-diabetic models treated with SAC (150 mg/kg) for 7 weeks ameliorated cognitive deficits through modulation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2)/(nuclear factor-kappa B (NF-κB)/toll-like receptor 4 (TLR4)/heme oxygenase 1 (HO-1), and acetylcholinesterase and attenuation of associated oxidative stress and neuroinflammation.
Naidu PB, Sathibadu Uddandrao VV, et al. 2016. Can J Diabetes. 40(5):442-8.	Diabetes was induced in models by streptozotocin (STZ) and nicotinamide (NA). SAC administration in models with diabetes showed effects similar to those of gliclazide in decreasing blood glucose, adlose reductase (AR), sorbitol dehydrogenase (SDH), sorbitol, fructose, glycosylated hemoglobin, thiobarbituric acid-reactive substances (TBARS) and hydroperoxides levels and significant increases in insulin, hemoglobin and glutathione (GSH) activity. Histopathologic studies also revealed the protective effect of SAC on pancreatic beta cells.

Antioxidative Effects of SAC

Imai J, Ide N, et al. 1994. Planta Med. 60:417-20.	SAC inhibited the emission of low level chemiluminescence and the early formation of thiobarbituric acid reactive substances (TBA-RS), whereas water extracts of raw and heat-treated garlic enhanced such emissions. Imai et al. suggested that SAC has antioxidative efficacy.
Ide N, Ryu K, et al. 1996. 2nd International Congress on Phytomedicine. Munich, Germany. Sep 11-14. Abst #SL-109. *Amagase H, Matsuura H, et al. 2000. Ch. 6. In: Phytochemicals ad Phytopharmaceuticals. AOCS Press.Champaign, IL, pp. 62-78.*	SAC decreased the emission of low level chemiluminescence (LLC) initiated by t-butyl hydroperoxide 33% at 5 mmol/L and 45% at 10 mmol/L. SAC also demonstrated radical and hydrogen peroxide scavenging activities *in vivo*.
Ide N, Matsuura H, et al. 1996. Phytother Res. 10:340-1.	SAC demonstrated a scavenging effect on hydrogen peroxide and also inhibited the chain oxidation induced by a hydrophilic radical initiator *in vitro*.
Ide N, Nelson AB, et al. 1997. Planta Med. 63(3):263-4.	SAC was shown to prevent copper, a potent antioxidant, from oxidizing low-density lipoprotein (LDL)

cholesterol in an *in vitro* system.

Ide N, Lau BHS. 1999. Drug Dev Industr Pharm. 25(5):619-24.

SAC was found to prevent lactate dehydrogenase (LDH) and depletion of glutathione (GSH) in pulmonary endothelial cells (PAEC) exposed to oxidized low-density lipoproteins (Ox-LDL) and dose-dependently inhibited Ox-LDL-induced peroxide release from PAEC. SAC was also found to scavenge hydrogen peroxide. Thus, SAC protected endothelial cells from Ox-LDL-induced injury by removing peroxides and preventing GSH depletion.

Hsu CC, Huang CN, et al. 2001. J Nutr. 134(1):149-52.

Five cysteine-containing compounds derived from garlic, including SAC, were added to drinking water at 1 g/L for a 4-week treatment while cysteine was used as a comparison. At the end of the treatment, glutathione (GSH) levels were higher ($p<0.05$) in the kidney and liver than in controls. SAC and other cysteine-containing compounds were also found to increase catalase and glutathione peroxidase (GPX) activities in the kidney and liver. When compared with the control and cysteine-treated groups, the cysteine-containing compounds were found to decrease iron (Fe^{2+})- and glucose-induced lipid oxidation in plasma, kidney and liver cholesterol levels in plasma and liver, while they increased levels of α-tocopherol in the liver, plasma and kidney ($p<0.05$).

Banerjee SK, Mukherjee PK, et al. 2003. Phytother Res. 17(2):97-106.

Various preparations of garlic, mainly AGE, have shown to have promising antioxidant potential. SAC, a major compound in AGE but not in raw garlic, has been reported to have powerful antioxidant and radical scavenging effects. This review touches on several of these areas.

Herrera-Mundo MN, Silva-Adaya D, et al. 2006. Neurosci Res. 56(1):39-44.

The effects of SAC on early behavioral alterations, striatal changes in superoxide dismutase activity, lipid peroxidation and mitochondrial dysfunction induced by the systemic infusion of 3-nitropropionic acid (3-NPA) to models. SAC given to models 30 minutes before 3-NPA prevented the hyperkinetic pattern by the toxin. 3-NPA alone produced decreased activities of manganese and copper/zinc-dependent superoxide dismutase, increased lipid peroxidation and mitochondrial dysfunction in the striatum. Pre-treatment of 3-NPA-injected models with SAC resulted in a significant prevention of all these markers.

Padmanabhan M, Prince PS. 2006. Toxicology. 224(1-2):128-37.

Cell membrane damage in myocardial infarction-induced models increased enzymatic leakage, lipid peroxidation and free radical formation. Oral pretreatment with SAC (100 mg/kg and 150 mg/kg) improved superoxide dismutase, catalase, glutathione reductase and ascorbic acid enzymatic activities. End measures of lipid peroxidation, thiobarbituric acid reactive substances (TBARS), were decreased with SAC. It was concluded that improvements were made in lipid peroxide markers and anti-oxidant status due to the anti-oxidant effects of SAC.

Perez-De La Cruz V, Gonzalez-Cortes C, et al. 2006. Brain Res Bull. 68(5):379-83.

The antioxidant properties of SAC were examined on lipid peroxidation and mitochondrial dysfunction induced by 3-nitropropionic acid (3-NPA), a neurotoxin. Concentrations of 3-NPA at 0.75-2.5 mM produced enhanced levels of lipid peroxidation while increasing concentrations of SAC (0.1-2 mM) which decreased the peroxidative effects of 3-NPA. SAC at 0.75 mM also prevented 3-NPA (1 mM)-induced mitochondrial dysfunction. It was determined that the protective actions of SAC on 3-NPA-induced lipid peroxidation and mitochondrial dysfunction are due to its antioxidant properties.

Padmanabhan M, Mainzen Prince PS. 2007. Life Sci. 80(10):972-8.

In models who suffered myocardial infarction due to prolonged myocardial ischemia showed improvement in mitochondrial enzyme activities when pretreated with SAC. Padmanabhan et al. suggest that this is due to SAC antioxidant qualities.

Cruz C, Correa-Rotter R, et al. 2007. Am J Physiol Renal Physiol. 293(5):F1691-8.

SAC was given to nephrectromized models (200 mg/kg, i.p.) every other day for 30 days. SAC reduced nitrotyrosine, poly(adenine dinucleotide phosphate [ADP]-ribose), the subunits of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase p22phox and gp91phox and increased superoxide dismutase activity, suggesting that the renoprotective effects of SAC is associated with its antioxidant properties and that it may be used to delay the progression of renal damage.

Medina-Campos ON, Barrera D, et al. 2007. Food Chem Toxicol. 45(10):2030-9.

It was found that SAC was able to scavenge concentration-dependently all the species assayed superoxide anion O_2^-, hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), and peroxynitrite anion ($ONOO^-$). When the ability of SAC to scavenge these species was compared to those of the reference compounds it was found that the efficacy of SAC (a) to scavenge O_2^-, H_2O_2, OH^-, and $ONOO^-$ was lower, (b) to scavenge HOCl was similar, and (c) to scavenge $^1O_2$ was higher. In addition, it was found that SAC was able to prevent potassium dichromate ($K_2Cr_2O_7$)-induced toxicity in renal epithelial ($LLC\text{-}PK_1$) cells in culture.

Kalayarasan S, Sriram N, et al. 2008. J Appl Toxicol. 28(7):908-19.

In potassium dichromate-induced apoptosis and oxidative stress in hepatocytes of models, administration

	of SAC restored liver marker enzymes such as aspartate transaminase, alanine transaminase and lactate dehydrogenase to near normal status. SAC also reversed the decrease of enzymic antioxidants (superoxide dismutase, catalase, glutathione peroxidase), non-enzymic antioxidants (vitamin C and vitamin E) and the levels of reduced glutathione, while decreasing lipid peroxidation (LPO) and reactive oxygen species in liver tissues. The expression of nuclear factor-E2 related factor 2 (Nrf2), was activated showing a promising role of Nrf2-mediated antioxidant defense of SAC against chromium toxicity.
Segoviano-Murillo S, Sanchez-Gonzales DJ, et al. 2008. Phytother Res. 22(6):836-40.	SAC was given to models subjected to right nephrectomy and induced by ischemia and reperfusion (IR). SAC was able to ameliorate the increase in blood urea nitrogen (BUN) and serum creatinine and decrease the structural damage induced by IR. This protective effect was associated with a decrease in the immunostaining for 4-hydroxy-2-nonenal (4-HNE), which concludes the antioxidant properties of SAC are involved in its protective effect on renal ischemia and reperfusion injury.
Arguello-Garcia R, Medina-Campos ON, et al. 2010. 58:11226-33.	The hypochlorous acid (HOCl) scavenging capacities of 10 garlic compound containing modifications in the thioallyl group were determined. This scavenging activity was enhanced by increasing the number of sulfur (S) atoms or by the alanyl group and decreased in the absence of the C=C bond or in the presence of a sulfoxide group in the thioallyl group. SAC and its corresponding sulfoxide alliin, showed the highest and lowest hypochlorous acid (HOCl)-scavenging capacities, respectively.
Wang Q, Qang XL, et al. 2010. Antioxid Redox Signal. 12(10):1155-65.	SAC, *S*-propyl-L-cysteine (SPC) and *S*-propargyl-L-cysteine (SPRC) were found to preserve superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in an acute myocardial infarction (MI) models and also tissue glutathione (GSH) levels while reducing the formation of the lipid peroxidation product malonaldehyde (MDA) in ventricular tissues. This study provides novel evidence that SAC, SPC and SPRC have cardioprotective effects in MI by reducing the deleterious effects of oxidative stress by modulating the endogenous levels of hydrogen sulfide (H_2S) and preserving the activities of antioxidant defensive enzymes.
Maldonado PD, Alvarez-Idaboy JR, et al. 2011. J Phys Chem B. 115(45):13408-17.	SAC was able to scavenge hydroxyl radical (•OH) and peroxyl radical (ROO•), in a concentration-dependent way. Such activity was significantly ameliorated when the allyl group was replaced by benzyl (*S*-benzylcysteine [SBC]) or propyl (*S*-propylcysteine [SPC]) groups. It was shown for the first time that SAC is able to scavenge ROO•.
Yu J, Feng L, et al. 2012. Placenta. 33(6):487-94.	Human first-trimester extravillous trophoblast (TEV-1) cells and human placental explants were separately exposed to SAC, hydrogen peroxide (H_2O_2) or a combination of SAC and H_2O_2. Co-treatment of H_2O_2 and SAC significantly decreased reactive oxygen species (ROS) productions and increased nitric oxide (NO), cyclic 3'5' guanosine monophosphate (cGMP) and endothelial NO (eNOS) levels compared to the H_2O_2 treated alone groups ($p<0.05$), which were all reverted back to near control levels. Furthermore, SAC treatment increased NO and cGMP level of TEV-1 cells and explants in a dose-dependent manner even at non-oxidative stress status ($p<0.05$). However, when the TEV-1 cells were cultured in the presence of NOS inhibitor L-NG-nitroarginine methyl ester (L-NAME) and NO donor sodium nitroprusside (SNP), additional SAC treatment still significantly increased the NO level in comparison with SAC non-treated group ($p<0.05$). From this study, SAC is hypothesized to be a potential drug for preeclampsia (PE) treatment.
Colin-Gonzalez AL, Santana RA, et al. 2012. Oxid Med Cell Longev. 907162.	Difference antioxidant mechanisms (scavenging of free radicals and prooxidant species, induction of antioxidant enzymes, activation of nuclear factor-E2 related factor 2 [Nrf2 factor], inhibition of prooxidant enzymes and chelating effects) involved in the protective actions of AGE and SAC were reviewed. In addition, the ability of SAC to activate Nrf2 factor—a master regulator of the cellular redox state is highlighted. Original data showing the ability of SAC to activate Nrf2 factor in cerebral cortex is included.
Asdaq SM. 2015. Evid Based Complement Alternat Med. 328545.	In models treated with AGE and SAC, there was a significant decline in elevated levels of triglyceride, total cholesterol, alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), malondialdehyde, glutathione peroxidase enzyme activity, total glutathione and oxidized glutathione in serum and inclined superoxide dismutase, catalase, ferric reducing/antioxidant powder, and total sulfhydryl values in liver tissue with reduction in thiobarbituric acid reactive species.
Colin-Gonzalez AL, Becerril H, et al. 2015. Life Sci. 135:165-72.	Models received SAC (100 mg/kg, i.p.) every day 30 minutes before being submitted to mild immobilization (restraint) stress. SAC was shown to exhibit preventive effects in the stressed group as it improved behavior, reduced lipid peroxidation and prevented the increase of glutathione-S-transferase (GST) and glutathione peroxidase (GPx) activities, suggesting that SAC blunted primary pro-oxidative

stimuli induced by restraint stress.

Colin-Gonzalez AL, Ali SF, et al. 2015. Neurochem Int. 89:83-91.

This update reviews the recent and refreshing evidence of the benefits of using SAC against toxic and pathological conditions. It has a broad spectrum of protective actions including antioxidant, redox modulatory and anti-inflammatory activities, accompanied by anti-apoptotic, pro-energetic and signaling capacities.

Bayraktar O, Tekin N, et al. 2015. Naunyn Schmiedebergs Arch Pharmacol. 388(3):327-35.

In models with lipopolysaccharide (LPS)-induced sepsis, SAC administration decreased the abnormal increases in alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and high-sensitivity C-reactive protein (hsCRP) compared to the sepsis group. In lung tissue, myeloperoxidase (MPO) activity and nitric oxide (NO) levels were decreased in by SAC application in sepsis + SAC group compared with sepsis group. In liver tissue, a decreased level of DNA fragmentation was noted in the sepsis + SAC group when compared with the sepsis group. SAC appears to ameliorate LPS-induced indicators of liver damage and suppressed the discharge of NO and MPO in lung tissue via its antioxidant properties.

Ogawa T, Kodera Y, et al. 2016. Sci Rep. 6:21611.

The garlic derived thioallyl compounds SAC and *S*-allylmercaptocysteine (SAMC) were shown to increase the lifespan and stress resistance in Caenorhabditis elegans and reduce accumulation of reactive oxygen species (ROS). They selectively induce SKN-1 (Nrf1/2/3 orthologue) targets involved in oxidative stress defense although, interestingly, their treatments do not facilitate SKN-1 nuclear accumulation, but slightly increased intracellular SKN-1 levels. The data also indicate that thioallyl structure and the number of sulfur atoms are important for SKN-1 target induction.

Orozco-Ibarra M, Munoz-Sanchez J, et al. 2016. Biol Res. 49:7.

The effect of AGE and SAC were assessed on cobalt chloride (CoCl2)-chemical hypoxia model in PC12 cells. Treatment with AGE and SAC decreased reactive oxygen species (ROS) and protected against CoCl2-induced apoptotic cell death which depended on the CoCl2 concentration and incubation time. SAC or AGE decreased the number of cells in the early and late stages of apoptosis, in which was associated with attenuation in hypoxia inducible factor (HIF-1α) stabilization, an activity not previously reported for AGE or SAC.

Dvorakova M, Heroutova I, et al. 2016. PeerJ. 4:e2280.

SAC (0.1, 0.5 and 1.0 mM) was shown to reduce levels of reactive oxygen species (ROS) in maturing oocytes significantly after 24 h (reduced by 90.33, 82.87 and 91.62%, respectively), and 48 h (86.35, 94.42 and 99.05%, respectively) cultivation, without leading to a disturbance of the standard course of meiotic maturation. Oocytes matured in the presence of SAC furthermore maintained reduced levels of ROS even 22 h after parthenogenic activation (reduced by 66.33, 61.64 and 57.80%, respectively). A growth of early embryo cleavage rate (increased by 33.34, 35.00 and 35.00%, respectively) was also demonstrated in these oocytes.

Sun YE, Wang WD. 2016. Cell Miol Biol (Noisy-le-grand). 62(7):85-9.

SAC was separated and identified from Allium sativum, and was reacted with 1-pyrenemethanol to obtain pyrene-labelled SAC (Py-SAC). The activity of Py-SAC and Vitamin C (VC) with oxygen radical absorbance capacity (ORAC) as index, the concentrations of Py-SAC and VC were 58.43 mg/L and 5.72 mg/L respectively to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 8.16 mg/L and 1.67 mg/L to scavenge •OH respectively. Compared with VC, the clearance rates of Py-SAC to scavenge DPPH were much higher, Py-SAC could inhibit hydroxyl radical.

Attenuated Ischemic Brain Damage

Numagami Y, Sato S, et al. 1996. Neurochem Int. 29(2):135-43.

When SAC was administered 30 minutes prior to ischemic insult, there was a significant decrease in ischemic damage. This was indicated by decreased water (swelling of the brain) in this middle cerebral artery occlusion model. In a global ischemia model, SAC decreased the amount of reactive oxygen species generated due to ischemia.

Numagami Y, Ohnishi ST. 2001. J Nutr. 131(3 Suppl):1100S-5S.

The efficacy of SAC was studied as a free radical scavenger using brain ischemia models. In a middle cerebral artery occlusion model, pre-ischemia administration of SAC improved (i) motor performance and (ii) memory impairment, and reduced (iii) water contents and (iv) the infarct size. In a transient global ischemia model, (i) the production of free radicals (alkoxyl radicals) as studied by electron paramagnetic resonance spectroscopy (EPR) was biphasic; the first peak occurring at 5 minutes and the second peak at 20 minutes after reperfusion. SAC did not attenuate the first peak but did on the second peak. (ii) The lipid peroxidation as estimated by thiobarbituric acid reactive substances (TBA-RS) increased significantly at 20 minutes after reperfusion. SAC decreased TBA-RS to the levels found without ischemia. These results suggest that SAC would have beneficial effects in brain ischemia and that the

major protective mechanism may be the inhibition of free radical-mediated lipid peroxidation.

Chun HS, Kim J-M, et al. 2003. FASEB J. 17(5):A760. Abst #457.2.

The protective effect of SAC on the ischemic damage is examined. Researchers used human neuroblastoma cell line SK-N-SH, and incubated it with or without SAC for 48 hours and exposed them to simulated ischemia (hypoglycemia and hypoxia) followed by simulated reperfusion (reoxygenation). SAC showed a neuroprotective effect against ischemia neuronal damage both *in vitro* and *in vivo*.

Kim KM, Lee JC, et al. 2006. Free Radic Res. 40(8):827-35.

SAC decreased the size of infarction after transient or global ischemic insults. While it did not alter the *N*-methyl-D-aspartate excitotoxicity, SAC significantly scavenged the endogenously or exogenously produced peroxynitrite ($ONOO^-$) and reduced $ONOO^-$ cytotoxicity. SAC also inhibited the activity of extracellular signal-regulated kinase (ERK) increased in cultured neurons exposed to oxygen-glucose deprivation. The results indicate that SAC exerts its neuroprotective effect by scavenging $ONOO^-$ and inhibiting the ERK signaling pathway activated during initial hypoxic/ischemic insults.

Garcia E, Limon D, et al. 2008. Free Radic Res. 42(10):892-902.

In 3 models exerting striatal toxicity, SAC was shown to prevent lipid peroxidation (LP) and mitochrondrial dysfunction (MD) in synaptosomal fractions from 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridinium-treated models, but without complete restoration of dopamine levels in the first model. In the second model, SAC prevented LP and MD in synaptosomes from models infused with 6-hydroxydopamine into the *substantia nigra pars compacta*, but again, without total reversion of depleted dopamine levels. In the third model, SAC prevented MD in synaptosomes injected with 3-nitropropionic acid, but failed to prevent LP. SAC also prevented the aberrant motor activity patterns evoked by the three toxins. Altogether, these results suggest that the antioxidant properties of SAC are responsible for partial or total preservation of neurochemical, biochemical and behavioral markers, indicating that pro-oxidant reactions underlie the neurotoxicity in these models.

Atif F, Yousuf S, et al. 2009. Brain Res. 1265:128-37.

In a transient middle cerebral artery occlusion (MCAO) model with ischemia/reperfusion (I/R)-induced mitochondrial dysfunctions, SAC significantly restored adenosine triphosphate (ATP) content and the activity of mitochondrial respiratory complexes, which were severely altered. A marked decrease in calcium swelling and decrease in cytochrome *c* release were observed as a result of SAC treatment. SAC also restored the status of mitochondrial glutathione (GSH) and glucose 6-phosphate dehydrogenase (G6-PD), significantly decreased mitochondrial lipid peroxidation (LPO), protein carbonyl (PC) and hydrogen peroxide (H_2O_2) content, significantly improved neurological deficits and significantly reduced brain edema.

Ashafaq M, Khan MM, et al. 2012. Nutr Res. 32(2):133-43.

Models were subjected to middle cerebral artery occlusion (MCAO) for 2 hour and 22-hour reperfusion. SAC was administered intraperitoneally 30 minutes before the onset of ischemia and after the ischemia at the interval of 0, 6 and 12 hours. After 24 hours, SAC treatment significantly reduced ischemic lesion volume, improved neurological deficits, combated oxidative loads and suppressed neuronal loss. Behavioral and biochemical alterations observed after MCAO were further associated with an increase in glial fibrillary acidic protein and inducible nitric oxide expression and were markedly inhibited by SAC treatment.

Shi H, Jing X, et al. 2015. J Neurochem. 133(2):298-308.

S-allyl cysteine (SAC) treatment resulted in an increase in nuclear factor erythroid-2-related factor 2 (Nrf2) protein levels and subsequent activation of antioxidant response element pathway genes in primary cultured neurons and models. Exposure of primary neurons to SAC provided protection against oxygen and glucose deprivation-induced oxidative insults. In wild-type ($Nrf2^{+/+}$) models, systemic administration of SAC attenuated middle cerebral artery occlusion-induced ischemic damage, a protective effect not observed in Nrf2 knockout ($Nrf2^{-/-}$) models. These findings provide the first evidence that activation of the Nrf2 antioxidant response by SAC is strongly associated with its neuroprotective effects against experimental stroke and suggest that targeting the Nrf2 pathway may provide therapeutic benefit for the treatment of stroke.

Anti-Cancer and Cancer-Preventive Effects of SAC

Thomson M, Ali M. 2003. Curr Cancer Drug Targets. 3(1):67-81.

Numerous studies have demonstrated the chemopreventive activity of garlic by using different garlic preparations including fresh garlic extract, AGE, garlic oil and a number of organosulfur compounds derived from garlic. Recent research has also focused on the antimutagenic activity of garlic. It has also been observed that AGE but not fresh garlic extract, exhibits radical scavenging activity. The two major compounds in AGE, SAC and *S*-allylmercaptocysteine (SAMC), have the highest radical scavenging ability. Because of this, consumption of garlic may provide protection from cancer development.

Borek C. 2005. JMHG. 2(3):346-52.

SAC and *S*-allylmercaptocysteine (SAMC) found in AGE have been shown to destroy cancer cells by inducing apoptosis, decrease the growth of prostate cancer cells by 80% while SAMC has also been shown to inhibit the growth of breast cancer cells, erythroleukemia and colon cancer cells.

Ameliorates Cardiotoxicity of Doxorubicin

Mostafa MG, Mima T, et al. 2000. Planta Med. 66(2):148-51.

Clinical uses of doxorubicin, a potent anticancer drug effective against a wide range of human neoplasms, have been limited due to its serious cardiotoxic effects, which are likely the results of generation of free radicals and lipid peroxidation. SAC has been reported to have antioxidant and radical scavenging effects. Thus, the effect of SAC on doxorubicin toxicity was examined. Severe doxorubicin toxicity was induced by a single intraperitoneal injection. SAC (30 mg/kg) was also injected intraperitoneally daily for 5 days, starting 2 days prior to the administration of doxorubicin. Doxorubicin injection induced a mortality rate of 58%, which SAC treatment reducing the doxorubicin-induced mortality rate to 30%. The severe body weight loss caused by doxorubicin (13%) was also significantly attenuated by SAC treatment (9%). Treatment with SAC significantly reduced the level of serum creatine phosphokinase. Histological analysis demonstrated that heart and liver damage was significantly less severe in SAC-treated models, than those receiving doxorubicin. These results suggest that SAC research may ultimately lead to a resolution of the adverse effects of doxorubicin treatment in cancer chemotherapy.

Inhibited the Growth of Carcinogen-Induced Tumors of the Breast

Amagase H, Milner J. 1992. FASEB J. 6(4):Abst #3329.

SAC (0.5 or 2.5 mg/kg diet) supplementation markedly depressed the occurrence of 7,12-dimethylbenz(a)anthracene (DMBA)-DNA adducts in mammary cells by 70% or 80%, respectively, but did not alter food intake or weight gain.

Amagase H, Milner J. 1993. Carcinogenesis. 14(8):1627-31.

SAC dose-dependently reduced the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA)-DNA adduct formation in mammary glands.

Tiwari RK, Pinto J, et al. 1993. Breast Cancer Res Treat. 27(1/2):Abst #80.

Li G, Qiao CH, et al. 1995. Oncol Rep. 2(5):787-91.

SAC and *S*-allylmercaptocysteine (SAMC) were found to inhibit the growth and proliferation of transformed human breast cells and increased both glutathione *S*-transferase (GST) and peroxidase levels in the non-transformed cells. GST is critical for detoxification and gene expression.

Liu JZ, Schaffer EM, et al. 1995. Experimental Biology. Abst #95.

SAC was shown to be an effective inhibitor of *N*-methylnitrosourea-induced mammary tumors. Final tumor incidence was 81% in control models and 38% in those fed SAC.

Milner JA, Schaffer EM, et al. 1996. 2nd International Congress on Phytomedicine. Munich, Germany. Sep 11-14. Abst #SL-110.

SAC is an effective inhibitor of chemically-induced transformation *in vitro* and *in vivo*. SAC reduced the formation of revertants of *Salmonella typhimurium* TA100 following exposure to nitrosomorpholine (NMOR), a known liver carcinogen. *N*-methyl-*N*-nitrosoura (MNU)-induced and 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumors were reduced by SAC.

Schaffer EM, Liu JZ, et al. 1996. Experimental Biology. FASEB J. Abst #96.

Schaffer EM, Milner JA. 1996. 1996. 87th Annual Meeting of American Association for Cancer Research. Washington, D.C. Apr 20-24. Abst #1924.

SAC inhibited the initiation of 7,12-dimethylbenz(a)anthracene (DMBA) mammary carcinogenesis. Selenium appeared to enhance the activity of SAC.

Schaffer EM, Liu J, et al. 1997. Cancer Lett. 102(1-2):199-204.

SAC and diallyl disulfide (DADS) are effective inhibitors of *N*-methyl-*N*-nitrosourea (MNU)-induced mammary carcinogenesis. Garlic powder, SAC and DADS supplementation significantly delayed the onset of mammary tumors compared to the control group. Tumor incidence 23 weeks after MNU treatment was reduced by 76%, 41% and 53% in models fed garlic, SAC and DADS, respectively. Also the quantity of mammary DNA alkylation occurring 3 hours after MNU treatment was reduced, specifically, O^6-methylguanine adducts were reduced by 27%, 18% and 23% for garlic powder, SAC and DADS respectively, and N^7-methylguanine 23% adducts decreased by 48%, 22% and 21%, respectively.

Song K, Milner JA. 1999. J Nutr. 129(3):657-61.

It was found that providing either 0.105 mmol diallyl disulfide (DADS) or SAC by gastric gavage thrice weekly for 2 weeks was effective in retarding 7,12-dimethylbenz(a)anthracene (DMBA) bioactivation. Isomolar alliin was not effective.

Gapter LA, Yuin OZ, et al. 2008. Biochem Biophys Res Commun. 2008. 367(2):446-51.

SAC significantly reduced anchorage-dependent and –independent growth of breast tumor cells in a dose- and time-dependent fashion and sub-lethal SAC-treatment altered mammary tumor cell adhesion and invasion through components of the extracellular matrix. Evidence provided suggests increased expression of E-cadherin and reduced matrix metalloproteinase-2 (MMP-2) expression and activity are partially responsible for inhibition of mammary tumor cell invasion by SAC.

Inhibited Proliferation of Prostate Cancer Cells

Chu Q, Ling MT, et al. 2006. Carcinogenesis. 27(11):2180-9.

Metastatic cancer is one of the main causes of cancer-related deaths since it rarely responds to available treatments. Using colony-forming, wound-closure as well as matrigel-invasion assays, it was found that the two main water-soluble constituents of garlic SAC and *S*-allylmercaptocysteine (SAMC), were able to suppress potentially invasive androgen-independent prostate cancer (Pca) cell proliferation and invasive abilities through restoration of E-cadherin expression in cancer cells.

Liu Z, Li M, et al. 2012. Mol Med Report. 5(2):439-43.

Androgen-independent human prostate cancer (PC-3) cells were incubated with SAC at three different concentrations. SAC suppressed the proliferation of PC-3 cells and led to cell cycle arrest at the Gap 0/Gap 1 (G0/G1) phases, as well as inducing cell apoptosis which was accompanied by the decreased expression of B-cell lymphoma 2 (Bcl-2) and increased expression of Bcl-2-associated X protein (Bax) and caspase 8.

Inhibited the Growth of Melanoma Cells

Takeyama H, Hoon DSB, et al. 1993. Oncology. 50(1):53-9.

SAC was found to inhibit the proliferation of nine human melanoma cell lines and one murine melanoma cell line in a dose-dependent manner. SAC inhibited cellular growth and proliferation and modulated major cell differentiation marker of melanoma.

Inhibited the Growth of Neuroblastoma Cells

Welch C, Wuarin L, et al. 1992. Cancer Lett. 63(3):211-9.

Welch et al. noted time- and dose-dependent inhibition of cell growth of human neuroblastoma cell (LA-N-5) cultures treated with SAC for 2 days.

Inhibited the Proliferation of Colon Cancer

Sumiyoshi H, Wargovich MJ. 1989. Proc Am Assoc Cancer Res. 30:181. Abst #718.

SAC significantly inhibited nuclear damage caused by the carcinogen dimethylhydrazine (DMH), thus decreasing the toxicity of this carcinogen. Both compounds significantly stimulated the activity of glutathione *S*-transferase (GST) in both the liver and colon. GST is an enzyme known to assist in the detoxification of carcinogens.

Sumiyoshi H, Wargovich MJ. 1990. Cancer Res. 50(16):5084-7.

The incidence and frequency of colon tumors induced by the carcinogen dimethylhydrazine (DMH) were significantly inhibited by pretreatment with SAC. SAC also stimulated the activity of glutathione *S*-transferase, an enzyme known to assist in the detoxification of carcinogens, in the liver and colon.

Hatono S, Jimenez A, et al. 1996. Carcinogenesis. 17(5):1041-4.

Aberrant crypt foci are considered to be the most likely precursors of colon cancer. SAC administration inhibited development in the colon of 1/3 to ½ of the foci induced by dimethylhydrazine (DMH) when given prior to this carcinogen (initiation phase). SAC was found to significantly enhance glutathione *S*-transferase (GST) activity not only in the liver but also in the proximal and middle small bowel. GST is a detoxification enzyme system in the body. Thus, SAC inhibited the development of pre-cancerous lesions in the colon and enhanced the activity of enzyme systems in the liver and small intestine, which detoxify carcinogens.

Knowles LM, Milner JA. 2001. J Nutr. 131(3 Suppl):1061S-6S.

Diallyl disulfide (DADS), a constituent in AGE and garlic oil, suppressed the proliferation of human colon tumor (HCT-15) cells by reducing apoptosis and altering cell division though a block in the Gap 2 phase/mitosis phase (G2/M) of the cell cycle. A marked suppression in the protein 34 ($p34^{cdc2}$) kinase activity and depressed protein tyrosine phosphatase (PTPase) activity accompanied the observed G2/M phase arrest. Western blot analysis revealed that 25 and 50 µM DADS decreased cdc25C phosphatase expression by 21% and 45%, respectively. DADS exposure caused a dose-dependent decrease in $p34^{cdc2}$ protein expression. Suppresion of $p34^{cdc2}$ and cdc25C expression likely accounts for the ability of DADS to inhibit $p34^{cdc2}$ kinase activity. Other sulfur compounds found in processed garlic may alter tumor cell proliferation by a similar mechanism.

Inhibited Lung Cancer Proliferation

Tang FY, Chiang EP, et al. 2010. J Agric Food Chem. 58:11156-64.

It was found that SAC significantly inhibited the proliferation of human non-small-cell lung carcinoma (NSCLC) A-549 cells and significantly suppressed the activation of mammalian target of rapamycin (mTOR), nuclear factor κB (NF-κB) and cyclin D1 molecules *in vitro*. Furthermore, SAC significantly inhibited the growth of highly metastatic human NSCLC cells in tumor-bearing models, indicating that SAC could effectively suppress the growth and malignant progression of human NSCLC and were associated with suppression of mTOR and NF-κB molecules *in vivo*.

Suppresses Hepatocellular Carcinoma

Ng KT, Guo DY, et al. 2012. PloS One. 7(2):e31655.

The proliferation rate and colony-forming abilities of a metastatic hepatocellular carcinoma (HCC) cell line (MHCC97L) cells were suppressed by SAC together with significant suppression of the expression of the proliferation markers, antigen Ki-67 and proliferating cell nuclear antigen (PCNA). Moreover, SAC hindered the migration and invasion of MHCC97L cells corresponding with up-regulation of E-cadherin and down-regulation of vascular endothelial growth factor (VEGF). Furthermore, SAC significantly reduced apoptosis and necrosis of MHCC97L cells through suppressing B-cell lymphoma-extra large (Bcl-xL) and B-cell lymphoma 2 (Bcl-2) as well as activating caspase-3 and caspase-9.

Chatterjee S, Patra D, et al. 2019. Environ Toxicol. 34(8):928-40.

SAC treatment demonstrated detailed molecular bagatelle associated with p38MAPK mediated effective suppression of cell growth both in HepG2 and chemically induced liver carcinoma. This study suggested significant contribution of p38MAPK-p53-DISC-Caspase pathway in the regulation of anti-neoplastic activity of SAC against hepatocellular carcinoma.

Suppresses Proliferation and Induces Apoptosis in Human Ovarian Cancer Cells

Xu YS, Feng JG, et al. 2014. Acta Pharmacol Sin. 35(2):267-74.

SAC (1-100 mmol/L) was shown to inhibit the proliferation of human epithelial ovarian cancer cell line A2780 cells *in vitro* in dose- and time-dependent manners (the IC_{50} value was approximately 25 mmol/L at 48 hours, and less than 6.25 mmol/L at 96 hours). Furthermore, SAC dose-dependently inhibited the colony formation of A2780 cells. SAC treatment resulted in G1/S phase arrest and induced apoptosis, accompanied by decreased expression of procaspase-3, Parp-1 and Bcl-2, and increased expression of active caspase-3 and Bax. SAC treatment also significantly reduced the migration of A2780 cells, and markedly decreased protein expression of Wnt5a, p-AKT and c-Jun, which were the key proteins involved in proliferation and metastasis.

Inhibited Oral Cancer Progression

Tang FY, Chiang EP, et al. 2009. J Nutr Biochem. 20(12):1013-20.

SAC dose-dependently inhibited te growth of human oral squamous cancer (CAL-27) cells and induced the expression of E-cadherin adhesion molecule. Other results revealed that SAC could restore the distribution of E-cadherin molecule on cell membrane, stabilized the adherent junction complex of E-cadherin/beta-catenin in oral cancer cells and significantly inhibited the activation of mitogen-activated protein kinases/extracellular signal-regulated kinases (MAPK/ERK) signaling pathway. These findings were associated with the down-regulation of the zinc finger protein (SLUG) or SNAI2 repressor protein.

Pai MH, Kyo YH, et al. 2012. Br J Nutr. 2011. 108(1):28-38.

SAC dose-dependently inhibited the growth of oral cancer in tumor-bearing models. The histopathological and immunohistochemical staining results indicated that SAC was able to effectively suppress tumor growth and progression of oral cancer *in vivo*.

Sustained Circulatory Antioxidants Depleted by Cancer-Causing Agent

Balasenthil S, Nagini S. 2000. J Biochem Med Biol Biophys. 4:35-9.

7,12-dimethylbenz(a)anthracene (DMBA) enhances lipid peroxidation in the circulation. In addition, it significantly depletes circulating antioxidants such as ascorbic acid, vitamin E, reduced glutathione and glutathione peroxidase. Administration of SAC significantly decreased DMBA-induced lipid peroxidation and enhanced the levels of antioxidants.

Sundaresan S, Subramanian P. 2003. Pol J Pharmacol. 55(1):37-42.

In a study where SAC was administered to *N*-nitrosodiethylamine (NEDA)-induced hepatocarcinogenesis, SAC was found to decrease tumor incidence and lipid peroxidation. SAC also increased antioxidant levels by decreasing the formation of free radicals.

Liver Protective Effects of SAC

Inhibited Both the Formation and Bioactivation of a Liver Carcinogen

Dion ME, Milner JA. 1996. FASEB J. 10(3):A498. Abst #2869.

SAC inhibited both the formation and bioactivation of the liver carcinogen nitrosomorpholine (NMOR). Adding SAC to a solution of sodium nitrite and morpholine prevented thse two compounds from generating nitrosomorpholine. SAC also prevented NMOR's ability to mutate a cell model.

Fukushima S, Takada N, et al. 2001. J Nutr. 131(3 Suppl):1049S-53S.

S-methylcysteine, a constituent in AGE, suppressed chemically-induced (sodium nitrite and morpholine) liver cancer.

Protected Liver Cells from the Liver Toxins: Paracetamol (Acetaminophen), Carbon Tetrachloride, Bromobenzene and Alcohol

Nakagawa S, Yoshida S, et al. 1985. Hiroshima J Med Sci. 34(3):303-9.

SAC was found to completely suppress the cytotoxicity of the potent liver toxin carbon tetrachloride (CCl_4), whereas four control drugs were found to be less effective at protecting the liver cells.

Nakagawa S, Kasuga S, et al. 1988. Phytother Res. 1(0):1-4.

SAC protected liver cells from the liver toxins, paracetamol and carbon tetrachloride. These compounds induce acute hepatitis. SAC appeared to enhance the activity of glutathione, a detoxifying enzyme, and act as chemical scavengers. Thus, SAC was found to be more effective than other chemicals used.

Wang BH, Zuzel K, et al. 1999. Toxicology. 132(2-3):215-25.

Pretreatment with SAC for several days, reduced bromobenzene (BB) toxicity in subsequently prepared liver slices. Up to 1 mM SAC was added to the culture medium of liver slices, suggesting this compound or a metabolite may be the glutathione (GSH)-sparing agent.

Hsu CC, Lin CC, et al. 2006. Food Chem Toxicol. 44(3):393-7.

Acetaminophen-induced depletion of glutathione (GSH) content in blood and organs could be lessened by SAC and *S*-propyl cysteine due to their antioxidant tendencies. Consequently, models demonstrated suppressed oxidation, inflammation and coagulation with improved liver function.

Kodai S, Takemura S, et al. 2007. Free Radic Res. 41(4):489-97.

SAC administered intraperitoneally (50-200 mg/kg) to models and was found to significantly suppress the increases of plasma alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) levels and hepatic total level of hydroxyoctadecadienoic acid (HODE), a new oxidative stress biomarker correlated with the amount of liver damage. SAC dose-dependently attenuated lipid peroxidation and increases in plasma malondialdehyde and hepatic 4-hydroxy-2-nonenal levels induced by carbon tetrachloride (CCl_4).

Yan SL, Yin MC. 2007. J Food Sci. 72(7):S511-5.

In alcohol-induced acute liver injury in models, preintake of SAC, *S*-ethyl cysteine (SEC), *S*-methyl cysteine (SMC) and *S*-propyl cysteine (SPC) was shown to significantly attenuate subsequent alcohol-induced lipid oxidation, glutathione (GSH) depletion and activity reduction of catalase and glutathione peroxidase ($P<0.05$); also attenuated were the alcohol-iduced elevation of C-reactive protein (CRP), interleukin-6 (IL-6), IL-10 and tumor necrosis factor-α (TNF-α) ($P<0.05$) and significantly retarded alcohol-induced cytochrome P450 2E1 (CYP2E1) activity increase ($P<0.05$). In the alleviate study, SAC, SEC, SMC and SPC restored liver GSH content ($P<0.05$); however, only SEC and SPC posttreatments significantly reduced lipid oxidation and alleviated alcohol-induced elevation of CRP, IL-6, IL-10 and TNF-α ($P<0.05$) and significantly diminished alcohol-induced CYP2E1 activity ($P<0.05$).

Kodai S, Takemura S, et al. 2015. J Clin Biochem Nutr. 56(3):179-85.

In models with liver cirrhosis induced by chronic carbon tetrachloride (CCl_4) administration, SAC administered from 4 weeks was shown to prevent the damaging changes (elevated plasma alanine aminotransferase, plasma lipid peroxidation, liver hydroxyproline, and liver transforming growth factor-β [TGF-β]) at 12 weeks. Furthermore, SAC was also shown to improve survival in a dose-dependent manner following consecutive CCl_4 administration.

Anti-Fibrotic Effects

Shinkawa H, Takemura S, et al. 2009. Osaka City Med J. 55(2):61-9.

Hepatic fibrosis was induced in models by porcine serum (PS) intraperitoneal injection. SAC (0.15% of basal diet) or *N*-acetylcysteine (NAC, 0.45% of basal diet) was orally administered for 12 weeks. SAC and NAC each markedly attenuated the development of hepatic fibrosis and suppressed the PS-induced

increase in α-smooth muscle actin (α-SMA) expressions, a marker of hepatic stellate cell (HSC) activation.

Tsukioka T, Takemura S, et al. 2017. Molecules. 22(4).

SAC was administered to pulmonary fibrosis-induced models by a single intratracheal instillation of bleomycin, as 0.15% SAC-containing diet from seven days prior to instillation of bleomycin (2.5 mg/kg) up until the conclusion of the experiment (14 days post-instillation). SAC significantly reduced collagen mRNA expression and protein deposition (33.3 ± 2.7 μg/mg and 28.2 ± 2.1 μg/mg tissue in vehicle- and SAC-treated models, respectively, and decreased fibrotic area. SAC also attenuated the increased expression of transforming growth factor-β1 (TGF-β1), in the models' lungs. While bleomycin instillation increased the number of myofibroblasts within the lung mesenchymal area, this was significantly reduced by SAC treatment.

Gong Z, Ye H, et al. 2018. Am J Transl Res. 10(5):1337-46.

Models were intraperitoneally injected with a mixture of carbon tetrachloride (CCl_4, 1 mL/kg body weight) and olive oil (1:1 v/v) every other day for 8 weeks to induce liver fibrosis. Treatment of SAC (50 mg/kg/day) attenuated CCl_4-induced liver fibrosis with anti-oxidant, anti-inflammatory and anti-fibrotic effects, and targeted STAT3/SMAD3 pathway to inhibit gene transcription.

Improves Non-Alcoholic Fatty Liver Disease in Type 2 Diabetes

Takemura S, Minamiyama Y, et al. 2013. J Clin Biochem Nutr. 53(2):94-101.

SAC was administered to models with nonalcoholic fatty liver disease for 13 weeks. SAC improved hemoglobin A1C, blood glucose, triglyceride, and low-density lipoprotein cholesterol levels. Furthermore, SAC normalized plasma insulin levels. SAC specifically activated the mRNA and protein expression of both peroxisome proliferator-activated receptor α and γ, as well as inhibiting pyruvate dehydrogenase kinase 4 in the liver. Sterol regulatory element-binding protein 1c and forkhead box O1 proteins were also normalized by SAC in the liver.

Brain, Neurotrophic, Anti-Aging and Anti-Depressant Effects of SAC

Numagami Y, Sato S, et al. 1996. Neurochem Int. 29(2):135-43.

When SAC was administered 30 minutes prior to ischemic insult, there was a significant decrease in ischemic damage. This was indicated by decreased water (swelling of the brain) in the middle cerebral artery occlusion model. In a global ischemia model, SAC decreased the amount of reactive oxygen species (ROS) generated due to ischemia.

Nishiyama N, Moriguchi T, et al. 1997. 2nd International Symposium on Natural Drugs. Maratea, Italy. Sep 28-Oct 1, p. 73.

SAC enhanced neuronal survival and branching of hippocampal culture studies. The authors suggested that it may be helpful in the development of therapeutic and/or prophylactic drugs for neurodegenerative disorders.

Moriguchi T, Matsuura H, et al. 1997. Neurochem Res. 22(12):1449-52.

SAC promoted the axonal branching of cultured neurons and neuronal survival.

Kosuge Y, Koen Y, et al. 2001. 74th Annual Meeting of the Japanese Pharmacological Society. Yokohama, Japan. Mar 21-23. Abst #P-849.

SAC selectively protects amyloid β (Aβ)-induced neuronal death in hippocampal neurons due to, at least in part, suppression of Aβ-induced oxidative stress. Mechanistic differences exist between Aβ-induced cell death in hippocampal neurons and in cerebellar granule neurons.

Peng Q, Buz'Zard AR, et al. 2002. Med Sci Monit. 8(8):BR328-37.

AGE and SAC were reported to decrease apoptosis by enhancing endogenous antioxidant defenses in a study determining their effects on amyloid β peptide (Aβ)-induced apoptosis and reactive oxygen species (ROS) generation in a pheochromocytoma of muroid adrenal medulla (PC12) cell line.

Chun HS, Kim J-M, et al. 2003. FASEB J. 17(5):A760. Abst #457.2.

An *in vitro* simulated ischemia model and an *in vivo* transient global ischemia model were used to study the protective effects of SAC. It was reported that SAC showed neuroprotective effects against *in vitro* and *in vivo* ischemia neuronal damage.

Ito Y, Kosuge Y, et al. 2003. Neurosci Res. 46(1):119-25.

Ito Y. 2004. Annual Meeting of the Korean Society of Applied Pharmacology. Seoul, Korea. Nov 11-12, pp. 124-8.

SAC protected neuronal cells against amyloid β-protein (Aβ)-induced cell death in a concentration-dependent manner and protected them against tunicamycin-induced neuronal death, which may be triggered by endoplasmic reticulum (ER) dysfunction in nerve growth factor (NGF)-differentiated pheochromocytoma of muroid adrenal medulla (PC12) cells.

Negi H, Arakawa M, et al. 2003. The 76th Annual Meting of the Japanese Pharmaceutical Society.

SAC was shown to aid in the protection of spinal neurons against glutamate neurotoxicity in organtypic spinal cultures (OSC). SAC may also aid in suppressing the loss of motor neurons induced by glutamate.

Kosuge Y, Koen Y, et al. 2003. Neurosci. 122(4):885-95.	SAC protected differentiated pheochromocytoma of muroid adrenal medulla (PC12) cells against amyloid β-protein (Aβ)- and tunicamycin-induced neuronal death and also attenuated the Aβ-induced increase of intracellular reactive oxygen species (ROS).
Kim JM, Chang N, et al. 2006. Biosci Biotechnol Biochem. 70(8):1969-71.	SAC, an active organosulfur compound derived from garlic, was found to reduce mortality with lesser incidence of stroke and also to lower overall stroke-related behavioral score in stroke-prone spontaneously hypertensive (SHRSP) models by dietary administration.
Kosuge Y, Sakikubo T, et al. 2006. Neurochem Int. 49(3):285-93.	Hippocampal neurons (HPN) were protected from neuronal cell death in the presence of SAC. In contrast, SAC did not exhibit any protective effects on cerebellar granule neurons (CGN). Both neurons and found in the endoplasmic reticulum (ER) are vulnerable to neuronal cell death with ER stress. Research has shown ER stress to be an important factor in amyloid β-peptide (Aβ)-induced neutotoxicity and Alzheimer's disease pathology.
Imai T, Kosuge Y, et al. 2007. Neuroscience. 147(3):639-51.	Amyloid β protein (Aβ) potentiation of tunicamycin-induced neurotoxicity was reversibly blocked by SAC and L-type calcium channel blocker nifedipine, in a restricted neuronal area of the muroid organotypic hippocampal slice cultures (OHCs). SAC, when simultaneously applied, also reversed the increases in calpain activity and the active forms of caspase-12 and caspase-13 by Aβ+TM with no change in the increased levels of glucose regulated protein (GRP)94, GRP78 and cytidine-cytidine-adenosine-adenosine-thymidine (CCAAT)/enhancer binding protein (C/EBP) homologous protein (CHOP). These results indicate that Aβ facilitates the calpain-caspase-12-caspase-13 pathway, thus potentiating TM-induced neuronal death in the hippocampus.
Ishige K, Takagi N, et al. 2007. J Pharmacol Sci. 104(1):46-55.	Organotypic hippocampal slice cultures (OHCs) were cultured for 7 weeks, SAC protected the cells in *Comu Ammonis* area 1 (CA1) and CA3 and the dentate gyrus from amyloid-β $(Aβ)_{25-35}$-induced toxicity. The increases in cleaved caspase-12 were also reversed by simultaneously applied SAC, suggesting that OHCs cultured for relatively longer periods are more susceptible to Aβ-induced toxicity and that the Aβ-induced cell death involves caspase-12-dependent pathways and SAC is able to protect against the Aβ-induced neuronal cell death through inhibition of the caspase-12-dependent pathway.
Elinos-Calderon D, Robledo-Arratia Y, et al. 2010. J Neural Transm. 117(1):35-44.	SAC was tested as a post-treatment in different *in vitro* and *in vivo* models. Quinolinic acid (QUIN) was used as a typical excitotoxic/pro-oxidant inducer, 3-nitropropionic acid (3-NP) was employed as a mitochondrial function inhibitor, and their combination (QUIN + 3-NP) was also evaluated in *in vitro* studies. For *in vitro* purposes, concentrations of SAC were added to isolated brain synaptosomes at different times after the incubation with toxins. For *in vivo* studies, SAC was given to QUIN- or 3-NP-strially lesioned models for 7 consecutive days. A differential pattern of protection was achieved by SAC, mostly expressed in the 3-NP toxic model, in which nerve ending protection was found within the first hour after the toxic insult started.
Garcia E, Villeda-Hernandez J, et al. 2010. Phytomedicine. 18(1):65-73.	Treatment of models with SAC for 5 days in parallel to 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridinium (MPTP) significantly reduced the degree of cell damage and prevented immunoreactivities of glial fibrillary acidic protein (GFAP), tumor necrosis factor-α (TNF-α) and inducible nitric oxide synthase (iNOS), as well as a reduced number of apoptotic nuclei. These results suggest that MPTP-induced morphological alterations recruit a pro-inflammatory component triggered by cytokine TNF-α release and nitric oxide formation, which is sensitive to the antioxidant properties of SAC. SAC is an effective experimental tool to reduce the brain lesions associated with oxidative damage and inflammatory responses.
Javed H, Khan MM, et al. 2011. Brain Res. 1389:133-42.	Models pre-treated with SAC (30 mg/kg) and vehicle (intraperitoneal; once daily for 15 days) were bilaterally injected with intracerebroventricular streptozotocin (ICV-STZ), whereas sham models received the same volume of vehicle. SAC pre-treatment prevented the cognitive and neurobehavioral impairments. Significant protection against an increased latency and path length observed in lesion, activities of reduced glutathione (GSH), glutathione peroxidase (GPx) and glutathione reductase (GR) in STZ group pre-treated with SAC. SAC pre-treated group also significantly attenuated the elevated level of thiobarbituric acid reactive substances (TBARS) and protected apoptotic parameters like DNA fragmentation, expression of B-cell lymphoma 2 (Bcl2) and protein 53 (p53).
Ray B, Chauhan NB, et al. 2011. J Neurochem. 117(3):388-402.	Significant neuroprotective and neurorescue properties of AGE and one of its ingredients, SAC, from reactive oxygen species (ROS) hydrogen peroxide (H_2O_2)-mediated insults to neuronal cells were observed. Treatment of AGE and SAC were found to protect neuronal cells when they were independently

co-treated with ROS. Furthermore, a novel neuropreservation effect of AGE was detected in that pre-treatment with AGE alone protected ~80% neuronal cells from ROS-mediated damage. AGE was also found to preserve synaptosomal-associated protein 25 (SNAP25) from ROS-mediated insult. For example, treatment with 2% AGE containing diet and SAC (20 mg/kg of diet) independently increased (~70%) levels of SNAP25 and synaptophysin in Alzheimer's amyloid precursor protein-transgenic models, of which the latter was significantly decreased in Alzheimer's disease (AD).

Tsai SJ, Chiu CP, et al. 2011. J Agric Food Chem. 59(11):6319-26.

In models with brain injury induced by D-galactose (DG), SAC, *S*-ethyl cysteine (SEC) and *S*-propyl cysteine (SPC) significantly decreased the production of amyloid-β (Aβ) peptide(1-40) and Aβ(1-42) and suppressed the expression of β-amyloid precursor protein (APP) and β-site APP cleavage enzyme 1 (BACE1) ($P<0.05$). Intake of SAC, SEC and SPC significantly retained protein kinase C (PKC) activity and the expression of PKC-α and PKC-γ ($P<0.05$), significantly lowered aldose reductase (AR) activity, AR expression and carboxymethyllysine (CML) and pentosidine levels ($P<0.05$), and significantly decreased reactive oxygen species (ROS) and protein carbonyl levels and restored brain glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase activities ($P<0.05$). These findings support that SAC, SEC and SPC via their anti-Aβ, antiglycative and antioxidative effects are potent agents against the progression of neurodegenerative disorders such as Alzheimer's disease.

Rojas P, Serrano-Garcia N, et al. 2011. J Nutr Biochem. 22(10):937-44.

Models were pretreated with SAC daily for 17 days, followed by administration of 1-methyl-4-phenylpyridinium (MPP^+). Models receiving SAC had significantly attenuated MPP^+-induced loss of striatal dopamine (DA) levels (32%). The neuroprotective effect of SAC against MPP^+ neurotoxicity was associated with blocked (100% of protection) of lipid peroxidation and reduction of superoxide radical production. Behavioral analyses showed that SAC improved MPP^+-induced impairment of locomotion (35%).

Ray B, Chauhan NB, et al. 2011. Curr Med Chem. 18(22):3306-13.

The effects of AGE and one of its active ingredients SAC in restricting several pathological cascades related to the synaptic degeneration and neuroinflammatory pathways associated with Alzheimer's disease (AD). Thus, based on the reported positive preliminary results reviewed herein, further research is required to develop the full potential of AGE and/or SAC into an effective preventive strategy for AD.

Garcia E, Santana-Martinez R, et al. 2014. Free Radic Res. 48(2):159-67.

SAC at 120 mg/kg, i.p., for 5 days in models partially ameliorated the 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridinium (MPTP) (30 mg/kg, i.p., for 5 days)-induced striatal and nigral dopamine and tyrosine hydroxylase depletion, attenuated the loss of Mn-SOD and HO-1 activities, and preserved the protein content of these enzymes. These findings suggest that SAC can exert neuroprotection since the origin of the dopaminergic lesion-at the substantia nigra (SN)-not only by means of direct antioxidant actions, but also through the Nrf2 nuclear transactivation and Phase 2 enzymes upregulation.

Imai T, Kosuge Y, et al. 2014. Amino Acids. 46(2):385-93.

SAC in AGE reversibly restored the survival of cultured model hippocampal neurons (HPNs) and increased the degradation of α-spectrin, a substrate of calpain, induced by tunicamycin, a typical ER stress inducer. Activities of μ- and m-calpains *in vitro* were also concentration dependently suppressed by SAC. SAC (1 mM) significantly reversed the effect of PD150606 at a concentration that elicited supramaximal inhibition (100 μM), but did not affect ALLN (1 nM)- and calpastatin (100 nM)-induced inhibition of μ-calpain activity. SAC has protective effects against ER stress-induced neuronal cell death attributable to suppression of calpain through interaction with its Ca^{2+}-binding site.

Zarezadeh M, Baluchnejadmojarad T, et al. 2016. Eur J Pharmacol. 795:13-21.

SAC mitigated lipopolysaccharide (LPS)-induced cognitive deficit in models via attenuation of oxidative stress, neuroinflammation, astroliosis, and acetylcholinesterase activity.

Denzer I, Münch G, et al. 2016. Food Chem. 194:843-8.

Preincubation with SAC and isoliquiritigenin increased mitochondrial membrane potential (MMP) in both pheochromocytoma cell (PC12) cell models of oxidative and nitrosative stress in a similar range as the positive control l-sulforaphane.

Zarezadeh M, Baluchnejadmojarad T, et al. 2017. Eur J Pharmacol. 795:13-21.

SAC was administered to models p.o. at doses of 25, 50, or 100 mg/kg/day, 30 minutes after lipopolysaccharide (LPS)-induced cognitive deficit, for seven days. SAC at 100 mg/kg/day improved spatial recognition memory in Y maze, discrimination ratio in novel object discrimination task, and retention and recall in passive avoidance test, mitigated lipid peroxidation marker malondialdehyde (MDA) and augmented superoxide dismutaste (SOD), catalase and glutathione (GSH) in hippocampal homogenate and lowered acetylcholinesterase activity. Meanwhile, SAC down-regulated nuclear factor κ B, toll-like receptor 4 (TLR4), glial fibrillary acidic protein (GFAP), and interleukin 1β (IL-1β) and up-regulated nuclear factor (erythroid-derived 2)-like 2 (Nrf2) in addition to lowering iba1-immunoreactive intensity in the hippocampus of LPS-injected group.

Zeinali H, Baluchnejadmojarad T, et al. 2017. Biomed Pharmacother. 97:557-63.

SAC was administered p.o. at a dose of 50 mg/kg/day to models immunized with myelin oligodendrocytic glycoprotein (MOG35-55). Results showed that SAC alleviated clinical signs and severity of multiple sclerosis (MS) and improved lumbar spinal cord tissue of tumor necrosis factor α (TNFα), interleukin 17 (IL-17), activity-dependent neuroprotector homeobox (ADNP), microtubule-associated proteins 1A/1 B light chain 3A (MAPILC3A), and matrix metalloproteinase 9 (MMP-9). SAC also attenuated inflammatory cell infiltration, axonal demyelination, and axonal loss in lumbar spinal cord in experimental autoimmune encephalomyelitis (EAE) group.

Franco-Eszastiga U, Santana-Martinez RA, et al. 2017. Neurochem Res. 42(11):3041-51.

SAC were administered to models for 90 days activated transcription factor related to NF-E2 (Nrf2) factor in the hippocampus (25-200 mg/kg for 24 hours, i.g.) and striatum (100 mg/kg) and significantly decreased p65 levels in the frontal cortex (25-200 mg/kg). On the other hand, SAC increased glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) activities mainly in the hippocampus and striatum. Finally, the hippocampus showed a major level of 8-hydroxy-2-deoxyguanosine (8-OhdG) compared with the striatum and frontal cortex.

Gomez CD, Aguilera P, et al. 2019. Adv Clin Exp Med. 28(12):1609-14.

In experimental models of cerebral ischemia, AGE and SAC treatments increased the main neuronal glucose transporter (GLUT3) and glutamate cysteine ligase catalytic subunit (GSLC) mRNA levels in control and under ischemic/reperfusion injury models. This suggests that AGE and SAC might induce neuroprotection, while controlling reactive oxygen species (ROS) levels, as indicated by the increase in GCLC expression, and regulating the energy content of the cell by increasing glucose transport mediated by GLUT3.

Other Protective Effects of SAC

Protected Cells from Toxic Carbon Tetrachloride in the Lungs

Mizuguchi S, Takemura S, et al. 2006. Biofactors. 26(1):81-92.

SAC was found to be effective at reducing carbon tetrachloride (CCl_4)-induced lung injury through intraperitoneal injection of CCl_4 into models twice a week for 8 weeks. SAC, *N*-acetyl cysteine (NAC) or L-cysteine (CYS) was orally administered everyday for 8 weeks. SAC significantly reduced the increases of transforming growth factor β, lipid peroxides, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma induced by CCl_4. SAC dose-dependently and significantly attenuated CCl_4-induced systemic inflammation and fibrosis of lung. SAC also prevented the decline of thiol levels, the increase of inducible nitric oxide synthase expression, the infiltration of leukocytes and the generation of reactive oxygen species (ROS) in lungs.

Protection Against Cyclophosphamide-Induced Bladder Hemorrhagic Cystitis

Bhatia K, Ahmad F, et al. 2008. Food Chem Toxicol. 46(11):3368-74.

SAC (150 and 300 mg/kg) was administered in cyclophosphamide (CP)-induced urotoxicity. SAC not only showed protection in tissue histology (lumen exudates, edema, vasodilation and hemorrhagic cystitis [HC]) but also improved the decreased activities of antioxidant enzymes, reduced lipid peroxidation (LPO) and increased glutathione (GSH) levels.

Abdi SA, Najmi AK, et al. 2016. Basic Clin Pharmacol Toxicol. 119(6):598-603.

Cyclophosphamide (CP) is the alkylating anticancer drug that induces a number of toxic effects including hemorrhagic cystitis (HC) in the urinary bladder. Uroplakins are unique urinary transmembrane proteins of urothelium, which may become potential targets of CP metabolites and reactive free radicals. SAC showed significant ($p<0.001$) protective effects against CP-induced alteration in mRNA levels and protein expression of uroplakin II and protected models from CP-induced HC. SAC was found to be more efficacious in affording protection in urinary bladder tissues than the thiol-rich drug mercaptoethane sulfonic acid (mesna).

Attenuates Renal Injury or Damage

Magendiramani V, Umesalma S, et al. 2009. J Appl Toxicol. 29(6):522-30.

Cyclosporine A (CsA)-induced nephrotoxicity in models. SAC administration improved renal function by significantly bringing out a decrease in peroxidative levels and increase in antioxidant status. SAC also reduced elevated expressions of inducible nitric oxide synthase (iNOS) and nuclear factor κB (NF-κB), moderately reduced an increase in the expression of matrix metalloproteinase-2 (MMP-2) and reduced an increase in the levels of serum constituent's urea, uric acid and creatinine in CsA-induced models. These results indicate that SAC has a protective action against CsA-induced nephrotoxity.

Khajevand-Khazaei MR, Azimi S, et al. 2019. Int Immunopharmacol. 69:19-26.

SAC was shown to be capable alleviate lipopolysaccharide (LPS)-induced acute liver injury (AKI) in an experimental model through mitigation of renal oxidative stress, inflammation, and apoptosis in addition to preservation of mitochondrial integrity and its favorable effect exhibits a dose-dependent pattern.

Sathibabu Uddandrao VV, Brahmanaidu P, et al. 2019. Eur J Nutr. 58(6):2425-37.

SAC showed adequate therapeutic effect against diabetic nephropathy (DN) by downregulation of inflammatory factors and attenuation of oxidative stress. Histological and scanning electron microscope (SEM) observations also indicated that SAC treatment notably reverses renal damage and protects the kidneys against hyperglycemia-mediated oxidative damage.

Restores Erectile Function in Diabetics

Yang J, Wang T, et al. 2013. Andrology. 1(3):487-94.

Reactive oxygen species (ROS) when produced in excess by an overactive nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system in penile tissue can cause erectile dysfunction (ED). Streptozotocin-induced diabetes model was given a 4-week treatment with insulin or SAC. Intracavernous pressure (ICP)/mean arterial blood pressure (MAP) was found to be markedly decreased in untreated diabetic models; SAC treatment restored the ratio to baseline (in non-diabetic untreated controls). SAC treatment normalized all the diabetic-induced effects, indicating that SAC treatment can restore erectile function in diabetic models by preventing ROS formation through modulation of NADPH oxidase subunit expression.

Mitigation of Doxorubicin-Induced Cardiotoxicity

Khan MA, Singh M, et al. 2013. Curr Clin Pharmacol. 9(3):288-97

Doxorubicin (DOX) is an effective and frequently used chemotherapeutic agents for various malignancies. However, its clinical use is hampered due to the development of cardiotoxicity. This paper reviews the prospect of herbal and botanical agents against DOX-induced cardiotoxicity with their proposed mechanisms including apoptosis, antioxidant potential, effect on mitochondria and calcium ion regulation, etc. A number of constituents with evident potential in prevention of DOX cardiotoxicity include SAC and others.

Prevents Cisplatin-Induced Nephrotoxicity and Oxidative Stress

Gomez-Sierra T, Molina-Jijon E, et al. 2014. J Pharm Pharmacol. 66(9):1271-81.

SAC (25 mg/kg) given to models prevented the cisplatin (CP)-induced renal damage and attenuated CP-induced decrease in nuclear-erythroid 2-related factor-2 (Nrf2) levels and increase in protein kinase C beta 2 (PKCβ2), nicotinamide adenine dinucleotide phosphate oxidase subunits (p47(phox) and gp91(phox)) expression in renal cortex and oxidative stress and decrease in the activity of catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) in proximal and distal tubules.

Protects the Retina Against Kainate Exitotoxicity

Chao HM, Chen IL, et al. 2014. Am J Chin Med. 42(3):693-708.

In vivo retinal excitotoxicity was induced by an intravitreous injection of 100 μM kainite into a model's eye for 1 day. The excitotoxicity-induced alterations were significantly blunted when 100 μM SAC and/or the other kainite receptor antagonist CNQX was applied. It seems SAC protects the retina against kainite excitotoxicity via an inhibition of the up-regulation of inducible nitric oxide synthase (iNOS) and matrix metalloproteinases-9 (MMPs-9) as well as a modulation of glial activation and apoptosis.

Attenuates Aminoglycoside-Induced Hearing Loss

Uzun L, Kokten N, et al. 2016. Clin Exp Otorhinolaryngol. 9(4):309-13.

Gentamicin is a potent aminoglycoside antibiotic in which ototoxicity and nephrotoxicity are the main side effects. Models injected with gentamicin and treated with either *S*-allylmercaptocysteine (SAMC) (Genta-w SAMC), diallyl disulfide (DD) (Genta-w DD), SAC (Genta-w SAC), gentamicin without any active compounds (AC) (Genta-w/o AC), or control. Using the brainstem evoked response audiometry (BERA) test, the mean amplitude of auditory thresholds (sensation level [SL]) for the groups were 22±8, 25±5, 30±9, 54±11, and 10±7 dB SL, respectively. The differences between every active compound group (Genta-w SAMC, Genta-w DD, and Genta-w SAC) and Genta-w/o AC were statistically significant ($P<0.016$).

Inhibits TNF-α-induced Inflammation in Keratinocytes

Basu C, Chatterjee A, et al. 2019.

Results of the study suggest that SAC inhibits tumor necrosis factor-α (TNF-α)-induced inflammation in

Exp Dermatol. 28(11):1328-35.

HaCaT keratinocyte cells via a combined effect entailing the inhibition of the p38 and the JNK pathways and NF-κB pathway via the sustained activation of ERK.

Reduces Inflammation and Mucus Overproduction in Allergic Asthma

Shin NR, Kwon HJ, et al. 2019. Int Immunopharmacol. 68:124-30.

In an ovalalbumin (OVA)-induced asthma model, SAC was shown to effectively suppress allergic airway inflammation and mucus production, which shows potential for use in treating allergic asthma.

SAC Has Been Confirmed to be Bioavailable and Active

Nagae S, Ushijima M, et al. 1994. Planta Med. 60(3):214-7.

SAC is a biologically active transformation product from garlic. SAC was rapidly and easily absorbed in the gastrointestinal tract and then distributed mainly in the plasma, liver and kidney after oral consumption. Its bioavailability was between 87.2% through 103% in models.

Rosen RT. 1999. 2nd International Conference on Food Factors (2nd IcoFF). Kyoto, Japan. Dec 12-17. Abst #SI4-1.

Rosen R. 2000. 3rd International Congress on Phytomedicine. Munich, Germany. Oct 11-13. 7(2):51. Abst #SL-105.

Rosen et al. established a procedure for measuring SAC in blood and urine of human subjects after consumption of garlic and garlic products. SAC was easily detected in plasma and urine by high performance liquid chromatography-mass spectrometry (HPLC-MS) using negative ion atmospheric pressure chemical ionization mode (ApcI)-MS. SAC levels in the blood reach levels of 800 parts per billion (ppb) in the blood after consumption of three AGE capsules.

Amagase H. 2000. 3rd International Congress on Phytomedicine. Munich, Germany. Oct 11-13. 7(2):51.

SAC is one of the water-soluble compounds in garlic derived from γ-glutamyl peptides that increases during extraction. SAC is utilized to standardize AGE and contended to be the only compliance marker compound for clinical studies involving garlic consumption. Since SAC is present in garlic preparations, has many biological effects and is confirmed to be bioavailable, it can be considered one of the active principles in garlic preparations.

Rosen R, Hiserodt R, et al. 2000. Biofactors. 13(1-4):241-9.

Allicin is decomposed in stomach acid into various allyl sulfides, disulfides and other volatiles. Thus, allicin is not detected anywhere.

Amagase H. 2001. FASEB J. 15(4):501.10. Abst #A633.

Amagase H. 2002. Ch. 15. In: Quality Management of Nutraceuticals. ACS Symposium Series 803. Washington, D.C., pp. 63-75.

Amagase stressed the importance of choosing appropriate, bioavailable marker compounds in standardizing herbs to assure quality and utility of the final product. Such markers can also assure compliance in clinical studies. For example, allicin is often thought to be an active ingredient for standardizing garlic products, though this compound is transient, actually not found in any product, and not bioavailable. SAC is a more appropriate choice since it has shown beneficial effects in many studies and is bioavailable. Numerous recent clinical studies, utilizing "allicin-standardized" products have shown a lack of efficacy, likely due to inappropriate standardization.

Kodera Y, Suzuki A, et al. 2002. J Agric Food Chem. 50:622-32.

Physical, chemical and biological properties of SAC were investigated. SAC showed stable properties under tested conditions and its acute/subacute toxicity was very minor. The pharmacokinetics of SAC was investigated after oral administration of garlic supplement containing SAC to human volunteers. SAC from garlic consumption, was rapidly absorbed from the gastrointestinal tract, however, the half-life and excretion time were more than 10 hours and 30 hours, respectively.

Amagase H. 2006. J Nutr. 136(3 Suppl):716S-25S.

This review article clarifies the real active compounds in garlic and different preparations. Not all of the active compounds of garlic are known and allicin-like transient compounds are not directly active. Ample research shows that an allicin-free garlic preparation, such as AGE, that is standardized with a bioavailable component, SAC, is active and various effects of garlic may be attributed to it. Various other chemicals constituents in garlic including non-sulfur compounds like saponins contribute to the essential biological activities of garlic.

SAC Has Confirmed Safety

Imada O. 1990. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. Aug 28-30, p. 47.

The median lethal dose (LD_{50}) for oral intake of SAC in models was 8890 mg/kg body weight in male models and 9390 mg/kg body weight in female models. Consequently, the toxicity of allicin was almost 30-fold to that of SAC, 309 mg/kg and 363 mg/kg body weight in male and female models, respectively. In subacute toxicological studies of SAC, pathological effects were not seen until 500 mg/kg.

Geng Z, Lau BHS. 1997. Free Radic Biol Med. 23(2):345-50.

The toxicity of SAC in cultures of human T cells and found that 0.5 to 2.0 mg/ml did not affect cell viability.

Pharmacokinetics

Nagae S, Ushijima M, et al. 1994. Planta Med. 60(3):214-7.

SAC is a biologically active transformation product from garlic. Nagae et al. found that SAC was rapidly and easily absorbed in the gastrointestinal tract and then distributed mainly in plasma, liver and kidney after oral consumption. Its bioavailability was between 87.2% through 103% in models.

Amano H, Kazamori D, et al. 2016. J Nutr. 146(2):456S-459S.

This study was designed to determine the overall pharmacokinetics of SAC in models. SAC was administered orally or intravenously to models at a dose of 5 mg/kg and was shown to be well absorbed with a bioavailability of 98%. Two major metabolites of SAC, *N*-acetyl-*S*-allylcysteine (Nac-SAC) and *N*-acetyl-*S*-allylcysteine sulfoxide (Nac-SACS), were detected in plasma but was markedly lower than those of SAC. Although some SAC was metabolized, most of the orally absorbed SAC was excreted into urine as its *N*-acetylated metabolites. The amounts of SAC, Nac-SAC, and Nac-SACS excreted in urine over 24 hours were 2.9%, 80%, and 11%, respectively. Low renal clearance of SAC indicated that it undergoes extensive renal reabsorption.

Chemistry

Amagase H, Rosen RT. 2004. International Congress on Natural Products Research. Jul 21-Aug 4.

Allicin is not active nor a marker compound in garlic products. AGE uses SAC since it is bioavailable and active in the body. It is reasonable to use this compound for standardization.

S-ALLYLMERCAPTOCYSTEINE (SAMC): A CONSTITUENT UNIQUE TO AGED GARLIC EXTRACT

Koch H. Lawson LD. 1996. In: Garlic the Science and Therapeutic Application of Allium sativum L. and Related Species. Baltimore, MD, p. 104.

Heber D. 1997. Am J Clin Nutr. 66(2):425-6.

Kodera Y. 1997. Ch. 11. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 95-103.

AGE contains a unique sulfur-containing compound known as *S*-allylmercaptocysteine (SAMC). SAMC is produced only during aging and is not present in fresh raw garlic or in various garlic preparations. SAMC is a water-soluble compound that has shown an array of effects, as seen in the following studies, including antioxidative, liver protective and anticarcinogenic effects.

Antioxidative and Radioprotective Effects of SAMC

Pedraza-Chaverri J, Barrera D, et al. 2004. BMC Clin Pharmacol. 4(5).

SAMC treatment was found to weaken the gentamicin-induced oxidative and nitrosative stress as well as the destruction to the kidneys (nephrotoxicity) *in vivo*. SAMC was also found to scavenge hydroxyl radicals and singlet oxygen *in vitro*.

Ogawa T, Kodera Y, et al. 2016. Sci Rep. 6:21611.

The garlic derived thioallyl compounds *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC) were shown to increase the lifespan and stress resistance in Caenorhabditis elegans and reduce accumulation of reactive oxygen species (ROS). They selectively induce SKN-1 (Nrf1/2/3 orthologue) targets involved in oxidative stress defense although, interestingly, their treatments do not facilitate SKN-1 nuclear accumulation, but slightly increased intracellular SKN-1 levels. The data also indicate that thioallyl structure and the number of sulfur atoms are important for SKN-1 target induction.

Uzun L, Kokten N, et al. 2016. Clin Exp Otorhinolaryngol. 9(4):309-13.

Gentamicin is a potent aminoglycoside antibiotic in which ototoxicity and nephrotoxicity are the main side effects. Models injected with gentamicin and treated with either SAMC (Genta-w SAMC), diallyl disulfide (DD) (Genta-w DD), *S*-allylcysteine (SAC) (Genta-w SAC), gentamicin without any active compounds (AC) (Genta-w/o AC), or control. Using the brainstem evoked response audiometry (BERA) test, the mean amplitude of auditory thresholds (sensation level [SL]) for the groups were 22±8, 25±5, 30±9, 54±11, and 10±7 dB SL, respectively. The differences between every active compound group (Genta-w SAMC, Genta-w DD, and Genta-w SAC) and Genta-w/o AC were statistically significant ($P<0.016$).

Zhu X, Jiang X, et al. 2017. Nutrients. 9(2).

Models were treated with cisplatin, a potent chemotherapeutic agent, with or without pre-treatment with SAMC. SAMC was confirmed to significantly attenuate cisplatin-induced renal damage. Pre-treatment with SAMC reduced nuclearfactor (NF)-κB activity, up-regulated nuclear factor erythroid 2-related factor 2 (Nrf2) and NAD(P)H:quinine oxidoreductase 1 (NQO1) expression and down-regulated inflammatory cytokine levels after cisplatin administration. Cisplatin-induced apoptosis in cultured human kidney (HK-2) cells were significantly attenuated by SAMC.

Protected Cells from Oxidant Injury

Ide N, Matsuura H, et al. 1996. Phytother Res. 10:340-1.

SAMC demonstrated a scavenging effect on hydrogen peroxide. It also inhibited the chain oxidation induced by a hydrophilic radical initiator. Hydrogen peroxide yields a free radical by reacting with iron or copper, a process called the Fenton reaction. This free radical damages both membranes and DNA and/or induced lipid peroxidation.

Ide N, Ryu K, et al. 1996. 2nd International Congress on Phytomedicine. Munich, Germany. Sep 11-14. Abst #SL-109.

Amagase H, Matsuura H, et al. 2000. Ch. 6. In: Phytochemicals and Phytopharmaceuticals. AOCS Press. Champaign, IL, pp. 62-78.

SAMC was found to decrease emissions of low level chemiluminescence (LLC) initiated by the oxidant t-butyl hydroperoxide in liver tissue. SAMC inhibited LLC emissions 60% at 5 mmol/L and 82% at 10 mmol/L, which is nearly the same effect as the potent antioxidant glutathione. SAMC also scavenged hydrogen peroxide *in vivo*.

Ide N, Lau BHS. 1997.

SAMC could inhibit copper-induced peroxidation of low-density lipoproteins (LDL) in a concentration-

Experimental Biology. FASEB J. New Orleans, LA. 11(3):A122. Abst #713.

dependent manner. Lipid oxidation was determined by measuring thiobarbituric acid reactive substances (TBA-RS). It was also found that when SAMC was preincubated with pulmonary artery endothelial cells, cell damage caused by oxidized LDL was prevented, as indicated by prevention of lactate dehydrogenase release, loss of cell viability and TBA-RS formation.

Liver Protective Effects of SAMC

In vivo Protection from Carbon Tetrachloride and Paracetamol

Nakagawa S, Yoshida S, et al. 1985. Hiroshima J Med Sci. 34(3):303-9.

SAMC completely suppressed the cytotoxicity of the potent liver toxin carbon tetrachloride (CCl_4), whereas four positive control drugs (vitamin E, piperonyl butoxide, glycyrrhizin and glutathione) were found to be less effective at protecting the liver cells.

Nakagawa S, Kasuga S, et al. 1988. Phytother Res. 1(0):1-4.

SAMC protected liver cells from the liver toxins paracetamol (acetaminophen) and carbon tetrachloride (CCl_4), which induce acute hepatitis. SAMC appeared to enhance the activity of glutathione, a detoxifying enzyme, and acted as chemical scavengers. SAMC was found to be more effective than other chemicals used.

Kodera Y. 1997. Ch. 11. In: Nutraceuticals: Designers Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 95-103.

Kodera suggested that the biotransformation products of SAMC to diallyl polysulfides in the liver might be responsible for the liver-protective effects of garlic which protect the liver from free radicals generated by carbon tetrachloride (CCl_4).

Sumioka I, Itakura Y, et al. 1997. 70th Annual Meeting of the Japanese Biochemical Society. Kanazawa, Japan. Sep 22-25.

The effect of SAMC or *N*-acetylcysteine (NAC) on acetaminophen (APAP)-induced liver injury was evaluated using a model system. SAMC was given 24 hours prior to and 2 hours after the administration of APAP and plasma alanine aminotransferase (ALT) activity, glutathione (GSH) contents and cytochrome P450 2E1 activity were determined. The data indicated that ALT activity was increased by APAP in plasma but was decreased with SAMC or NAC. SAMC and NAC both had an effect on the GSH contents of the liver, which indicates liver protection; however, the mode of action may be different between these compounds.

Sumioka I, Matsuura T, et al. 1998. Jpn J Pharmacol. 78(2):199-207.

SAMC given to models 2 and 24 hours before administration of acetaminophen (APAP) prevented liver damage as shown by a reduction in alanine aminotransferase (ALT) activity that is enhanced by APAP. ALT was shown to decrease by 79%, 97% and 100% when APAP was given in conjunction with 50, 100 and 200 mg/kg SAMC. SAMC also prevented the reduction in glutathione induced by APAP administration. One mechanism proposed for liver protection was inhibition of cytochrome P450 2E1 activity since SAMC suppressed an enzyme representative of P450 2E1 activity. Cytochrome P450 2E1 is a major enzyme responsible for the bioactivation of APAP. SAMC pretreatment also suppressed the increase in hepatic lipid peroxidation and the decrease in liver reduced Coenzyme Q9 (CoQ_9H_2), suggesting an antioxidant effect.

Sumioka I, Matsuura T, et al. 1999. 10th Annual Meeting on Chogoku/Shikoku Biological Radical Association. Okayama, Japan. Jul 30. Abst #7.

The effect of SAMC on acetaminophen (APAP)-induced liver injury was evaluated using a model system. SAMC was given 24 hours before and 2 hours after APAP administration and plasma alanine aminotransferase (ALT) activity, lipid peroxidation in liver, α-tocopherol, CoQ10 and glutathione (GSH) contents were determined. Administration of APAP increased ALT activity in plasma 72-fold and lipid peroxidation 2.9-fold after 6 hours. However, SAMC was able to return both ALT activity and lipid peroxidation back to normal levels while improving cytochrome P450 2E1 (CYP2E1) in a dose-dependent manner. It is determined that SAMC protects liver function through its antioxidant effects.

Sumioka I, Matsuura T, et al. 1999. 72nd Annual Meeting of the Japanese Biochemical Society. Yokohama, Japan. Oct 6-9. Abst #4P-218.

In order to elucidate the mechanism by which SAMC shows liver protection from induced acetaminophen (APAP)-induced liver injury, the effects of SAMC on cytochrome P450 2E1 (CYP 2E1), uridine diphosphate-glucuronyltransferase (UDP-GT) and sulfotransferase (ST) activities were determined. SAMC improved the P450 2E1 activity but had no effect on UDP-GT or ST. This data suggests that SAMC inhibits APAP-induced liver injury through suppression of CYP 2E1 activity.

Sumioka I, Matsuura T, et al. 2000. 41st Annual Meeting of the Japanese Biochemical Society. Yonago, Japan. Apr 21-22. Abst #21.

The effect of SAMC on acetaminophen (APAP)-induced liver injury was evaluated using a model system. APAP was given 30 minutes prior to SAMC administration and plasma alanine aminotransferase (ALT) activity, glutathione (GSH) contents, cytochrome P4502E1 (CYP2E1) activity and the expression of inducible 70 kilodalton heat shock protein (HSP70i), a stress protein, were determined. ALT activity was increased by APAP in plasma but was decreased by SAMC. To further understand this mechanism, GSH contents in the liver and CYP2E1 activities were determined. No change was seen in GSH contents of the

liver by SAMC but SAMC improved the activity enhanced by APAP and HSP70i. HSP70i is enhanced by a complex metabolite of APAP which is *N*-acetyl-*p*-benzoquinone imine (NAPQI), and high molecule substances. Therefore, this data indicates that SAMC treatment inhibits APAP-induced liver injury by reducing NAPQI production through the inhibition of CYP2E1 activity.

Xiao J, Liong EC, et al. 2012. Eur J Nutr. 5(3):323-33.

Models were intraperitoneally injected with carbon tetrachloride (CCl_4) to induce acute hepatotoxicity with or without pre-treatment of SAMC intraperitoneal injection. After 8 hours, SAMC was shown to reduce CCl_4-triggered cellular necrosis and inflammation in the liver under histological analysis. Co-treatment of SAMC and CCl_4 enhanced the expressions of antioxidant enzymes, reduced the nitric oxide (NO)-dependent oxidative stress, and inhibited lipid peroxidation induced by CCl_4. Administration of SAMC also ameliorated hepatic inflammation induced by CCl_4 via inhibiting the activity of nuclear factor-κB (NF-κB) subunits p50 and p65, thus reducing the expressions of pro-inflammatory cytokines, mediators and chemokines, as well as promoting pro-regenerative factors at both transcriptional and translational levels.

Protective Effect in Non-Alcoholic Fatty Liver Disease

Xiao J, Ching YP, et al. 2013. Eur J Nutr. 52(1):179-91.

Models fed with a diet with high unsaturated fat (30% fish oil) for 8 weeks developed non-alcoholic fatty liver disease (NAFLD) with or without an intraperitoneal injection of 200 mg/kg SAMC three times per week. Co-treatment of SAMC attenuated NAFLD-induced liver injury, fat accumulation, collagen formation and free fatty acids (FFAs). SAMC decreased the lipogenesis marker, restored the lipolysis marker, and reduced the expression levels of pro-fibrinogenic factors and diminished liver oxidative stress partly through the inhibition in the activity of cytochrome P450 2E1-dependent pathway. SAMC treatment also partially mitigated NAFLD-induced inflammation via reduction of pro-inflammatory mediators, chemokines and suppressor of cytokine signaling. SAMC also restored altered phosphorylation status of FFAs-dependent MAP kinase pathways and diminished in the nuclear transcription factors (NF-κB and AP-1) activity during NAFLD development.

Anti-Cancer and Cancer-Preventive Effects of SAMC

Thomson M, Ali M. 2003. Curr Cancer Drug Targets. 3(1):67-81.

Numerous studies have demonstrated the chemopreventive activity of garlic by using different garlic preparations including fresh garlic extract, AGE, garlic oil and a number of organosulfur compounds derived from garlic. Recent research has also focused on the antimutagenic activity of garlic. It is also been observed that AGE, but not fresh garlic extract, exhibits radical scavenging activity. The two major compounds in AGE, *S*-allylcysteine (SAC) and SAMC have the highest radical scavenging ability. Because of this, consumption of garlic may provide protection from cancer development.

Borek C. 2004. Int Cancer Ther. 3(4):333-41.

Borek C. 2004. J Nutr. 134(11):3207S-9S.

SAMC induces apoptosis in human prostate cancer cells and in breast cancer cells, as well as colon cancer cells by activating caspase-3, inhibiting antiapoptotic protein B-cell lymphoma 2 (Bcl-2) and disrupts microtubules in cancer cells preventing further growth.

Borek C. 2005. JMHG. 2(3):346-52.

S-allyl cysteine (SAC) and SAMC found in AGE have been shown to destroy cancer cells by inducing apoptosis, decrease the growth of prostate cancer cells by 80% while SAMC has also been shown to inhibit the growth of breast cancer cells, erythroleukemia and colon cancer cells.

Inhibited the Growth of Carcinogen-Induced Tumors of the Breast

Tiwari RK, Pinto J, et al. 1993. Breast Cancer Res Treat. 27(1/2):Abst #80.

Li G, Qiao CH, et al. 1995. Oncol Rep. 2(5):787-91.

SAMC inhibited the growth and proliferation of transformed human breast cells. They also increased both glutathione *S*-transferase and peroxidase levels in the non-transformed cells.

Inhibited Carcinogen-Induced Precancerous Activity of the Lungs

Wang K, Wang Y, et al. 2016. Int Immunopharmacol. 34:37-43.

SAMC was shown to inhibit benzo(a)pyrene (B(a)P) carcinogenesis in human lung cells (A549 cell line) through mechanisms that include suppression of cell proliferation, cell cycle regulation, attenuation of reactive oxygen species (ROS) formation, inhibition of DNA damage, increase of superoxide dismutase (SOD) activity and inhibition of nuclear factor-kappa B (NF-κB) activity.

Inhibited the Growth of Colon/Colorectal Cancer Cells

Knowles LM, Milner JA. 1997. FASEB J. 11(3):A422. Abst #2445.

Xiao D, Pinto J, et al. 2002. FASEB J. 17(4):A1152. Abst #711.1.

Xiao D, Pinto JT, et al. 2003. Cancer Res. 63(20):6875-37.

Experimental carcinogenesis studies indicate that components of garlic (i.e., allyl sulfides) inhibit both the initiation and promotion stages of tumorigenesis for various types of cancer, including colorectal. It was previously reported that SAMC inhibits growth, arrest cells in Gap 2 phase/mitosis phase (G_2/M) and induces apoptosis in human colon cancer cells. This study concludes that the garlic-derived compound SAMC exerts antiproliferative effects by binding directly to disrupting the microtubule (MT) assembly, thus arresting cells in mitosis and triggering c-Jun NH_2-terminal kinase (JNK) and caspase-3 signaling pathways that lead to apoptosis.

Shirin H, Pinto JT, et al. 2001. Cancer Res. 61(2):725-31.

SAMC inhibited the growth of two human colon cancer cell lines SW-480 and HT-29 at doses similar to that of sulindac sulfide (SS), a well-established colon cancer chemopreventive agent. SAMC also induced apoptosis, caused a marked increase in endogenous levels of reduced glutathione and augmented the growth inhibitory and apoptotic effects of SS when co-administered.

Ross SA, Finley JW, et al. 2006. J Nutr. 136(3 Supp):852S-4S.

Models were provided a semi-purified, casein-based diet with or without 57 or 570 µmol/kg of *S*-allyl cysteine (SAC), diallyl disulfide (DADS) or SAMC for 13 weeks prior to determination of aberrant crypt foci (ACF) and aberrant crypt number. All treatments, except 57 µmol/kg SAC, significantly lowered ACF compared to controls. ACF was significantly reduced by DADS and SAMC at both concentrations tested. This study revealed that all allyl sulfur compounds are not equivalent in retarding early preneoplastic markers for colon cancer.

Liang D, Qin Y, et al. 2011. Cancer Lett. 310(1):69-76.

SAMC could effectively suppress the growth and metastasis of colorectal cancer (CRC) cells both *in vivo* and *in vitro*. The anticancer effect of SAMC was related to the decreased proliferation and increased apoptosis as well as necrosis of cancer cells. Taken together, the proliferation and metastasis of CRC cells can be significantly suppressed by SAMC treatment under both *in vitro* and *in vivo* conditions, thus, SAMC may be a promising candidate for CRC chemotherapy.

Li S, Yang G, et al. 2017. Oncol Rep. 38(3):1637-44.

The xenografting HCT-116 cancer cells in models, the combination therapy of rapamycin and SAMC had enhanced tumor-suppressing ability with the upregulation of the Bax/Bcl-2 ratio as a consequence of activated apoptosis, inhibition of autophagic activity and prevention of Akt phosphorylation. The rapamycin and SAMC combination activated antioxidant transcription expressions of nuclear factor erythroid 2-related factor 2 (Nrf2) and downstream gene NAD(P)H:quinine oxidoreductase 1 (NQO1). Cocomitantly, autophagosome cargo p62 was downregulated.

Suppressed the Proliferation and Metastasis of Ovarian Cancer Cells

Wu J, Zhao S, et al. 2016. Exp Mol Pathol. 100(2):294-302.

In vivo and *in vitro* experiments using three ovarian cancer cell lines were subjected to SAMC treatment. SAMC suppresses both the proliferation and distant metastasis of epithelial ovarian cancer cells, and insensitivity to one of the cell lines to SAMC was closely related to the high level of urviving expression and that combination of SAMC treatment together with urviving knockdown might be a potential strategy for treating certain variants of ovarian cancers.

Inhibited the Growth of Prostate Cancer Cells

Sigounas G, Hooker J, et al. 1997. Nutr Cancer. 27(2):186-91.

SAMC in AGE could inhibit the growth of hormone responsive prostate cancer cell line CRL-1740. Even at 0.05 mM, SAMC totally suppressed the growth of CRL-1740 cells compared with the solvent-treated control cells.

Pinto J, Qiao C, et al. 1997. FASEB J. 11(3):A439. Abst # 2541.

Pinto JT, Qiao CH, et al. 2000. The Prostate. 45(4):304-14.

SAMC may inhibit the growth of androgen responsive human prostate cancer cells (LNCaP). Testosterone, a specific androgen or male hormone, enhances the activity or growth of LNCaP. SAMC wsa found to enhance the catabolism or degradation of testosterone. Therefore, it was suggested that SAMC by catabolizing testosterone, hampered the progression or activation of these cancer cells. Prostate specific antigen (PSA) levels were markedly reduced after treatment with SAMC, a marker for prostate cancer.

Chu Q, Ling MT, et al. 2006. Carcinogenesis. 27(11):2180-9.

Metastatic cancer is one of the main causes of cancer-related deaths since it rarely responds to available treatments. Using colony-forming, wound-closure as well as matrigel-invasion assays, two main water-soluble constituents in garlic, *S*-allyl cysteine (SAC) and SAMC, were able to suppress potentially invasive prostate cancer (Pca) cell proliferation and invasive abilities through restoration of E-cadherin

expression in cancer cells.

Howard EW, Ling MT, et al. 2007. Clin Cancer Res. 13(6):1847-56.

SAMC was shown not only to inhibit the growth of primary tumors by up to 71% ($P<0.001$) in androgen-independent prostate cancer model, but also reduced the number of lung and adrenal metases by as much as 85.5% ($P=0.001$) without causing notable toxicity. The metastatic suppression was accompanied by a 91% reduction of viable circulating tumor cells ($P=0.041$), suggesting that SAMC prevents the dissemination by decreasing tumor cell intravasation.

Inhibited the Growth of Bladder Cancer Cells

Hu H, Zhang XP, et al. 2011. Mol Med Report. 4(1):9-16.

Studies have shown that expression of inhibitor of differentiation (Id-1) is increased in bladder cancer and is associated with drug resistance. Results of this study show that overexpression of Id-1 was shown to reduce the positive effect of SAMC on cell survival, while inactivation of Id-1 increased cellular susceptibility to SAMC. Other tests confirmed the apoptosis of bladder cancer cells induced by SAMC was shown to be negatively regulated by Id-1 expression. The inhibitory effect of SAMC on the invasion and migration of bladder cancer cells was found to be associated with the down-regulation of Id-1. Results demonstrated that SAMC-induced apoptosis is associated with the Id-1 pathway, and that the inactivation of Id-1 enhances the ability of SAMC to inhibit the survival, invasion and migration of bladder cancer cells.

Inhibited the Growth of Erythroleukemia Cell Lines

Sigounas G, Hooker J, et al. 1997. Nutr Cancer. 27(2):186-91.

SAMC in AGE could inhibit the growth of two erythroleukemia cell lines HEL and OCIM-1. HEL cells showed complete suppression of growth at ≥ 0.25 mM SAMC and even at 0.1 mM SAMC inhibited HEL cell growth by > 70%. OCIM cels exhibited a 55% reduction in growth at 0.1 mM SAMC.

Sigounas G, Hooker J, et al. 1997. Nutr Cancer. 28(2):153-9.

SAMC was further confirmed to inhibit the growth of two erythroleukemia cell lines HEL and OCIM-1. It induced a dose-dependent inhibition with a 50% lethal dose of 0.046 mM for OCIM-1 cells and 0.093 mM for HEL cells. From analyses of [^{3}H] thymidine incorporation and high molecular weight DNA fragmentation, Sigounas et al. concluded that SAMC is an effective antiproliferative agent against erythroleukemia cells that induces death by apoptosis.

Lea MA, Randolph VM, et al. 2002. International Research Conference on Food, Nutrition and Cancer. Washington, D.C. Jul 11-12. J Nutr. 132:3549S.

Allyl sulfur compounds found in AGE have been determined to inhibit histone acetylation and cell growth. Garlic extracts with thiosulfinate inhibited DS19 murine erythroleukemia cell proliferation. DS19 cells incubated with SAMC or allyl isothiocyanate produced similar degree of downregulation of both histone deacetylase (HDAC) and histone acetyltransferase (HAT) activities.

Apoptosis of Gastric Cancer Cells

Lee Y, Kim H, et al. 2011. Biol Pharm Bull. 34(5):677-81.

SAMC was found to induce apoptosis in gastric cancer cells *in vitro*. SAMC was reported to inhibit tumor growth rate by 31.36% and 37.78% at doses of 100 and 300 mg/kg, respectively. The apoptosis index of 100 mg/kg and 300 mg/kg of SAMC was 20.87 ± 2.50% and 30.61 ± 2.42%, respectively. For control, 100 mg/kg SAMC and 300 mg/kg SAMC, the positive rate of B-cell lymphoma 2 (bcl-2) protein expression were 15.20 ± 1.67%, 10.94 ± 1.57% and 8.24 ± 1.07% and the positive rate of bcl-2-associated X (bax) protein expression were 15.30 ± 1.90%, 23.18 ± 1.81% and 25.26 ± 3.03%, respectively. Decreases in bcl-2 messenger RNA (mRNA) and increases in bax mRNA by SAMC in a dose-dependent manner by reverse transcription-polymerase chain reaction (RT-PCR).

Yan JY, Tian FM, et al. 2013. Eur Rev Med Pharmacol Sci.

Human gastric cancer cells line (SGC 7901) was cultured with different concentrations of SAMC. SAMC at 300 μM induced SGC 7901 apoptosis through cell viability. Polymerase chain reaction (PCR) assay demonstrated that JNK and P38 pathway played an important role.

Zhu X, Jiang X, et al. 2017. Biochem Biophys Res Commun. 491(3):821-6.

Human gastric cancer SGC-7901 cells were inoculated subcutaneously in models. When xenograft tumors reached about 100 mm^3, models were treated with SAMC for 30 days. SAMC administration effectively delayed the growth of of SGC-7901 xenografts withouts signs of toxicity. TUNEL staining confirmed that the tumors from SAMC-treated models exhibited a markedly higher apoptotic index.

OTHER CONSTITUENTS IN AGED GARLIC EXTRACT

Oil-Soluble and Water-Soluble Organosulfur Compounds

Weinberg DL, Manier ML, et al. 1992. J High Resolut Chromatogr. 15:641-54.

Weinberg DL, Manier ML, et al. 1993. J Agric Food Chem. 41:37-41.

AGE is rich in water-soluble compounds and contains small amounts of oil-soluble compounds. Weinberg et al. developed a methodology to detect, identify and quantify nine oil-soluble organosulfur compounds in AGE: allyl sulfide, allyl disulfide (diallyl disulfide), allyl trisulfide, allyl methyl sulfide, allyl methyl disulfide, allyl methyl trisulfide, methyl disulfide, methyl trisulfide and ethyl 2-propenesulfinate. It was found that the concentration of most of these constituents increased with time. Some compounds nearly tripled in concentration and other increased by an order of magnitude. The results were confirmed by an independent study of the extract from the National Cancer Institute.

Gwilt P, Lear CL, et al. 1994. Cancer Epidemiol Biomarkers Prev. 3(2):155-60.

Dimitrov NV, Bennink MR. 1997. Ch. 21. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 199-202.

Gwilt et al. and Dimitrov et al. also quantified various organosulfur compounds in AGE.

Vijayaraghavan M, Wanibuchi H, et al. 2000. Jpn J Cancer Res. 91(8):780-5.

The water-soluble components of garlic *S*-methylcysteine (SMC) and cysteine were shown to provide inhibitory effects on diethylnitrosamine (DEN)-induced hepatocarcinogenesis, along with sodium phenobarbitol (NaPB), at the promotion stage. However, only SMC was able to significantly reduce ornithine decarboxylase (ODC) enzyme activity.

Ichikawa M, Ide N, et al. 2006. J Agric Food Chem. 54(5):1535-40.

Ichikawa et al. developed and validated a simple, rapid and precise analytical method to determine seven organosulfur compounds that are found in garlic: alliin, isoalliin, methiin, cycloalliin, γ-L-glutamyl-*S*-methyl-L-cysteine (GSMC), γ-L-glutamyl-*S*-(2-propenyl)-L-cysteine (GSAC) and γ-L-glutamyl-*S*-(trans-1-propenyl)-L-cysteine (GSPC). The method consisted of using a one-step sample preparation procedure by high performance liquid chromatography, with overall recoveries of all seven organosulfur compounds of 97.1 to 102.3%.

Kodera Y, Ushijima M, et al. 2017. Molecules. 22(4). pii: E570.

S-1-Propenyl-L-cysteine (S1PC) is a stereoisomer of *S*-allyl-L-cysteine (SAC), an important sulfur-containing amino acid that plays a role for the beneficial pharmacological effects of AGE. Although the existence of S1PC in garlic preparations has been known since the 1960's, there was no report regarding the biological and/or pharmacological activity of S1PC until 2016. A series of studies were performed to examine the chemical, biological, pharmacological and pharmacokinetic properties of S1PC. S1PC existed only in trace amounts in raw garlic, but its concentration increased almost up to the level similar of SAC through aging process of AGE. S1PC showed immunomodulatory effects in vitro and in vivo, reduced blood pressure in a hypertensive model was readily absorbed after oral administration in models with bioavailability of 88-100%. Additionally, S1PC had little inhibitory influence on human cytochrome P450 activities, even at a concentration of 1 mM.

Fujii T, Matsutomo T, et al. 2018. J Agric Food Chem. 66(40):10506-12.

γ-Glutamyl-*S*-allylmercaptocysteine (GSAMC), a putative precursor compounds of *S*-allylmercaptocysteine (SAMC), was isolated and identified from AGE. The change of their contents in AGE during the aging process was analyzed chronologically 1 month to 22 months. SAMC content reached a maximum at approximately 4 months, whereas GSAMC content reached a maximum at 1 month and then decreased during the subsequent aging period. Using model reactions, SAMC was produced from GSAMC by the garlic protein fraction having γ-glutamyl transpeptidase (GGT) activity, and its production was suppressed by a GGT inhibitor. Furthermore, the production of GSAMC from allicin and γ-glutamyl-*S*-allycysteine (GSAC)/γ-glutamyl-*S*-1-propenylcysteine (GS1PC) was found in another model reaction. The reaction between allicin and GS1PC was faster than that between allicin and GSAC, and thus may be involved in the production of GSAMC in the early stage of aging process.

Yamaguchi Y, Kumagai H. 2020. Exp Ther Med. 19(2):1528-35.

S-allyl-L-cysteine sulfoxide (ACSO) is an odor precursor in garlic bulbs. The characteristics, biosynthesis, decomposition, metabolism and functions of ACSO are discussed further in detail in this review.

Lipid-/Cholesterol-Lowering Effect

Lee Y, Yeh Y-Y. 2003. FASEB J. 17(4):A752. Abst #455.1.

Water-soluble organosulfur compounds in Kyolic: *S*-allylcysteine (SAC), *S*-ethyl cysteine (SEC), *S*-propyl cysteine (SPC) and γ-glutamyl *S*-alk(en)yl cysteines, have shown maximal inhibition on cholesterol synthesis requires a concerted action of these various compounds in human hepatocellular carcinoma (HepG2) cells.

Lin CC, Yin MC. 2007. Br J Nutr. 2007. 99(1):37-43.

The intake of *N*-acetyl cysteine (NAC), *S*-ethyl cysteine (SEC) or *S*-propyl cysteine (SPC) treatment significantly decreased triacylglycerol (TAG) and total cholesterol contents ($P<0.05$) via enhancing the activity and messenger RNA (mRNA) expression of malic enzyme, fatty acid synthase and 3-hydroxy-3-methylglutaryl coenzyme A reductase ($P<0.05$).

Other Cardiovascular Effects

Matsutomo T, Ushijima M, et al. 2017. J Chromatogr B Analyt Technol Biomed Life Sci. 1046:147-55.

Treatment with AGE (2 g/kg body weight) or *S*-1-propenylcysteine (S1PC) (6.5 mg/kg body weight; equivalent to AGE 2 g/kg body weight) in spontaneously hypertensive models significantly decreased the systolic blood pressure (SBP) of models after the repeated administration for 10 weeks. After the treatment for 10 weeks, the plasma samples were analyzed and results indicated that 30 endogenous metabolites wer changed by the S1PC treatment. Furthermore, regression analysis showed correlation between SBP and the plasma levels of betaine, tryptophan and 3 LysoPCs.

Ushijima M, Takashima M, et al. 2018. J Pharm Pharmacol. 70(4):559-65.

A single oral administration of S-1-propenylcysteine (S1PC) (6.5 mg/kg BW) for 24 h significantly decreased the systolic blood pressure of spontaneously hypertensive model approximately 10% at 3 h after administration, and thereafter, the systolic blood pressure gradually returned to the baseline level in 24 h. Furthermore, S1PC significantly increased the blood flow at 3 h after administration at the dose of 6.5 mg/kg BQ.

Tsuneyoshi T, Kunimura K, et al. 2019. Nitric Oxide. 84:22-9.

In human umbilical vein endothelial cells (HUVECs), *S*-1-propenylcysteine (S1PC) was shown to augment degradation of BTB domain and CNC homolog 1 (BACH1) and heme oxygenase 1 expression in a nitric oxide (NO)-dependent manner, thereby suggesting that S1PC may be used for the treatment of various inflammatory diseases.

Immunomodulatory Effects

Suzuki J, Yamaguchi T, et al. 2016. Nutrition. 32(7-8):884-9.

In vitro study: model splenic lymphocytes were treated with *S*-1-propenylcysteine (S1PC) (0.1 and 0.3 mM) for 3 days were found to enhance intestinal immunoglobulin A (IgA) production in culture. *In vivo* study: Models were orally administered S1PC (7.5, 15, and 30 mg/kg) for 5 days. S1PC increased the IgA level and number of IgA-producing cells in Peyer's patches. Furthermore, S1PC induced the expression of X-box binding protein 1 (Xbp1) mRNA, an inducer of plasma cell differentiation, in Peyer's patches, accompanied by the degradation of paried box protein 5 and the activation of mitogen activated protein/extracellular signal-regulated kinase signaling pathway.

Anti-Inflammatory Effects

Suzuki JI, Kodera Y, et al. 2018. Sci Rep. 8(1):14148.

S-1-propenylcysteine (S1PC) in AGE inhibited toll-like receptor (TLR)-mediated interleukin-6 (IL-6) production by inducing the degradation of adaptor protein MyD88. S1PC was shown to directly denature MyD88 and induce the formation of protein aggregates. Consequently, MyD88 was degraded by aggresome-autophagy pathway.

Tsuneyoshi T, Kunimura K, et al. 2019. Nitric Oxide. 84:22-9.

In human umbilical vein endothelial cells (HUVECs), *S*-1-propenylcysteine (S1PC) was shown to augment degradation of BTB domain and CNC homolog 1 (BACH1) and heme oxygenase 1 expression in a nitric oxide (NO)-dependent manner, thereby suggesting that S1PC may be used for the treatment of various inflammatory diseases.

Antioxidative Effects

Chen CM, Yin MC, et al. 2007. Nutrition. 23(7-8):589-97.

Pre-intake of *N*-acetyl cysteine (NAC), *S*-ethyl cysteine (SEC), *S*-methyl cysteine (SMC) and *S*-propyl cysteine (SPC) significantly attenuated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced glutathione loss, retained the activity of glutathione peroxidase (GPX) and superoxide dismutase, diminished oxidative stress and suppressed MPTP-induced elevation of interleukin-6 (IL-6) and tumor

necrosis factor-α (TNF-α) (P<0.05) in models. The four cysteine-containing compounds also significantly elevated GPX mRNA expression and diminished TNF-α messenger RNA (mRNA) expression (P<0.05), improved MPTP-induced dopamine depletion and increased dopamine/3,4-dihydroxyphenylacetic acid content (P<0.05), in which these results suggest that NAC, SEC, SMC and SPC could provide antioxidative and anti-inflammatory protection for the striatum against the development of Parkinson's disease.

Wang Q, Qang XL, et al. 2010. Antioxid Redox Signal. 12(10):1155-65.

S-allyl-L-cysteine (SAC), *S*-propyl-L-cysteine (SPC) and *S*-propargyl-L-cysteine (SPRC) were found to preserve superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in an acute myocardial infarction (MI) models and also tissue glutathione (GSH) levels while reducing the formation of the lipid peroxidation product malonaldehyde (MDA) in ventricular tissues. This study provides novel evidence that SAC, SPC and SPRC have cardioprotective effects in MI by reducing the deleterious effects of oxidative stress by modulating the endogenous levels of hydrogen sulfide (H_2S) and preserving the activities of antioxidant defensive enzymes.

Uzun L, Kokten N, et al. 2016. Clin Exp Otorhinolaryngol. 9(4):309-13.

Gentamicin is a potent aminoglycoside antibiotic in which ototoxicity and nephrotoxicity are the main side effects. Models injected with gentamicin and treated with either *S*-allylmercaptocysteine (SAMC) (Genta-w SAMC), diallyl disulfide (DD) (Genta-w DD), *S*-allylcysteine (SAC) (Genta-w SAC), gentamicin without any active compounds (AC) (Genta-w/o AC), or control. Using the brainstem evoked response audiometry (BERA) test, the mean amplitude of auditory thresholds (sensation level [SL]) for the groups were 22±8, 25±5, 30±9, 54±11, and 10±7 dB SL, respectively. The differences between every active compound group (Genta-w SAMC, Genta-w DD, and Genta-w SAC) and Genta-w/o AC were statistically significant (P<0.016).

Inhibited the Activity of Human Cytochrome (CYP) Enzymes

Greenblatt DJ, Leigh-Pemberton RA, et al. 2006. J Nutr. 136(3 Suppl):806S-9S.

Two water-soluble components of AGE, *S*-methyl-L-cysteine and *S*-allyl-L-cysteine, at 100 µmol/L reduced cytochrome-P450 (CYP) isoform 3A (CYP3A) activity to 20-40% of control.

Inhibited the Levels of Hepatic CYP2E1 Protein

Wargovich MJ. 1998. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. Nov 15-17, p. 32.

Organosulfur compounds in alliums (AOSC) are effective in inhibiting carcinogenesis at the initiation stage of esophageal and colon cancers. Inhibitory effects are primarily due to AOSC's ability to reduce cytochrome P450 2E1 (CYP2E1) enzyme which activates carcinogens nitrosomethylbenzylamine and azoxymethane responsible for inducing esophageal and colon cancers.

Yang CS, Chhabra SK, et al. 2001. J Nutr. 131(3 Suppl):1041S-5S.

Garlic compounds diallyl sulfide (DAS) and diallyl sulfone ($DASO_2$) have proven effective in inhibiting chemical toxicity and carcinogenesis by competing for cytochrome P_{450} 2E1 (CYP2E1) enzyme, protecting against hepatotoxicity of acetaminophen and inhibiting the bioactivation of tobacco carcinogen 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK). However, these beneficial effects have only been observed with higher concentrations of garlic. Studies still need to be conducted using concentrations equivalent to dietary consumption and supplementation.

Wargovich MJ. 2006. J Nutr. 136(3 Suppl):832S-4S.

Research results indicate that two organosulfur compounds diallylsulfide (DAS) and allylmethylsulfide, are effective in significantly reducing levels of hepatic cytochrome p4502E1 (CYP2E1) protein.

Reduced the Incidence of Chemically-Induced Cancers

Ross SA, Finley JW, et al. 2006. J Nutr. 136(3 Supp):852S-4S.

Garlic and its associated allyl sulfur compounds have been shown to reduce the incidence of chemically-induced breast, colon, skin, uterine, esophagus and lung cancers. Aqueous suspensions and high exposure to *S*-allyl cysteine (SAC) have been shown to inhibit early stage colon cancer. All treatments significantly lowered colon cancer model compared to controls (p<0.05). This study reveals that all allyl sulfur compounds are not equivalent in retarding early stage of colon cancer.

Inhibit the Growth of Colon Cancer Cells

Ban JO, Yuk DY, et al. 2007. J Pharmacol Sci. 104(4):374-83.

Thiacremonone, a sulfurcompound isolated from garlic, was shown to inhibit colon cancer cell (SW620 and HCT116) growth followed by induction of apoptosis in a dose-dependent manner. Thiacremonone also modulated tumor necrosis factor-α (TNF-α) and tetradeanoyl phorbol acetate (TPA)-induced nuclear factor-κB (NFκB) transcriptional and DNA binding activity. Moreover, thiacremonone suppressed NFκB

target anti-apoptotic genes (B cell lymphoma 2 [Bcl-2], baculoviral inhibitor of apoptosis repeat-containing protein ½ [cIAP1/2] and X-linked inhibitor of apoptosis protein [XIAP]) and inflammatory genes (inducible nitric oxide synthase [iNOS] and cyclooxygenase-2 [COX-2]), whereas it induced apoptotic genes (B-cell lymphoma-2-associated X protein [Bax], cleaved caspase-3 and cleaved poly ADP-ribose polymerase [PARP]) expression. These results suggest that a novel sulfurocompound from garlic inhibited colon cancer cell growth through induction of apoptotic cell death by modulating of NFκB.

Inhibited Tumor Activity Against Primary Effusion Lymphoma

Shigemi Z, Furakawa Y, et al. 2016. Int J Oncol. 48(1):293-304.

Diallyl trisulfide (DAT) inhibited nuclear factor-κB (NF-κB) signaling and induced apoptosis through destabilization of tumor necrosis factor receptor associated factor 6 (TRAF6) in primary effusion lymphoma (PEL), a subtype of non-Hodgkins's B-cell lymphoma, *in vitro* and *in vivo*.

Liver Protective Effect

Yan SL, Yin MC. 2007. J Food Sci. 72(7):S511-5.

In alcohol-induced acute liver injury in models, preintake of *S*-allyl cysteine (SAC), *S*-ethyl cysteine (SEC), *S*-methyl cysteine (SMC) and *S*-propyl cysteine (SPC) was shown to significantly attenuate subsequent alcohol-induced lipid oxidation, glutathione (GSH) depletion and activity reduction of catalase and glutathione peroxidase ($P<0.05$); also attenuated were the alcohol-induced elevation of C-reactive protein (CRP), interleukin-6 (IL-6), IL-10 and tumor necrosis factor-α (TNF-α) ($P<0.05$) and significantly retarded alcohol-induced cytochrome P450 2E1 (CYP2E1) activity increase ($P<0.05$). In the alleviate study, SAC, SEC, SMC and SPC restored liver GSH content ($P<0.05$); however, only SEC and SPC posttreatments significantly reduced lipid oxidation and alleviated alcohol-induced elevation of CRP, IL-6, IL-10 and TNF-α ($P<0.05$) and significantly diminished alcohol-induced CYP2E1 activity ($P<0.05$).

Lin CC, Yin MC. 2007. Br J Nutr. 2007. 99(1):37-43.

The intake of *N*-acetyl cysteine (NAC), *S*-ethyl cysteine (SEC) or *S*-propyl cysteine (SPC) treatment significantly suppressed high saturated fat-induced hepatic messenger RNA (mRNA) expression of sterol regulatory element-binding protein (SREBP)-1c and SREBP-2 ($P<0.05$), increased hepatic glutathione content ($P<0.05$), restored the activity and mRNA expression of glutathione peroxidase, and alleviated the high saturated fat-induced oxidative stress ($P<0.05$).

Shinkawa H, Takemura S, et al. 2009. Osaka City Med J. 55(2):61-9.

Hepatic fibrosis was induced in models by porcine serum (PS) intraperitoneal injection. *S*-allyl cysteine (SAC) (0.15% of basal diet) or *N*-acetylcysteine (NAC, 0.45% of basal diet) was orally administered for 12 weeks. SAC and NAC each markedly attenuated the development of hepatic fibrosis and suppressed the PS-induced increase in α-smooth muscle actin (α-SMA) expressions, a marker of hepatic stellate cell (HSC) activation.

Chen LY, Chen Q, et al. 2016. Int Immunolpharmacol. 36:23-30.

In a model of alcohol administration and human normal liver cell line cultured with suitable ethanol to mimic the pathological condition of alcoholic fatty liver (AFL), administration of diallyl trisulfide (DATS) significantly lowered the accumulation of intracellular reactive oxygen species (ROS), but antioxidant capacity was increased by DATS. DATS also inhibited heptocyte apoptosis via down-regulating Bax expression and up-regulating Bcl-2 expression, and attenuated alcohol-induced caspase-dependent apoptosis. Lastly, SATS was found to increase the expressions of cystathionine gamma-lyase (CSE) and cystathionine beta-synthase (CBS), the major H2S-producing enzymes.

Chen LY, Chen Q, et al. 2016. Biomed Pharmacother. 79:35-43.

Diallyl trisulfide (DATS) was shown to prevent ethanol-induced injury, as indicated by the reduced activities of aspartate transaminase (AST) and alanine aminotransferase (ALT) in serum of models given a single dose of ethanol *in vivo*, and in culture medium in ethanol-stimulated LO2 cells (*in vitro*) to mimic alcoholic fatty liver (AFL), and inhibition of cell apoptosis. DATS reduced hepatic steatosis and alleviated ethanol-induced oxidative stress.

Brain and Neurotrophic Effects

Tsai SJ, Chiu CP, et al. 2011. J Agric Food Chem. 59(11):6319-26.

In models with brain injury induced by D-galactose (DG), *S*-allyl cysteine (SAC), *S*-ethyl cysteine (SEC) and *S*-propyl cysteine (SPC) significantly decreased the production of amyloid-β (Aβ) peptide(1-40) and Aβ(1-42) and suppressed the expression of β-amyloid precursor protein (APP) and β-site APP cleavage enzyme 1 (BACE1) ($P<0.05$). Intake of SAC, SEC and SPC significantly retained protein kinase C (PKC) activity and the expression of PKC-α and PKC-γ ($P<0.05$), significantly lowered aldose reductase (AR) activity, AR expression and carboxymethyllysine (CML) and pentosidine levels ($P<0.05$), and

significantly decreased reactive oxygen species (ROS) and protein carbonyl levels and restored brain glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase activities ($P<0.05$). These findings support that SAC, SEC and SPC via their anti-Aβ, antiglycative and antioxidative effects are potent agents against the progression of neurodegenerative disorders such as Alzheimer's disease.

Imai T, Kosuge Y, et al. 2016. J Pharmacol Sci. 130(3):185-8.

Various *S*-allyl-l-cysteine (SAC) derivatives were synthesized and tested for their effects on endoplasmic reticulum-induced neurotoxicity in cultured hippocampal neurons (HPNs). *S*-propyl-l-cysteine (SPC) exhibited the strongest neuroprotective activity in HPNs, followed by *S*-ethyl-l-cysteine (SEC) and *S*-methyl-l-cysteine (SMC).

Manral A, Meena P, et al. 2016. Neurotox Res. 30(3):407-26.

Diallyl disulfide (DADS) analogues 7k and 7l significantly inhibited Aβ1-42-induced neuronal cell death by inhibiting reactive oxygen species (ROS) generation in SH-Sy5Y human neuroblastoma cells. Moreover, they prevented apoptosis, in response to ROS, by restoring normal Bax/Bcl-2 ratio. Furthermore, it was observed that scopolamine-induced memory impairment in a model was coupled by alterations in neurotransmitters, acetylcholinesterase activity and oxidative stress markers. Administration of 7k and 7l at 5 mg/kg significantly reversed scopolamine-induced behavioral, biochemical, neurochemical and histological changes in a manner comparable to standard donepezil.

Potential in the Treatment of Osteoporosis

Chen Y, Sun J, et al. 2016. Int J Mol Sci. 17(9):1516.

Alliin (*S*-allyl-L-cysteine sulfoxides, SACSO), a major component of AGE, had an inhibitory effect in osteoclasteogenesis with dose-dependent manner via blocking the c-Fos-NFATc1 signaling pathway. Alliin also decreased the generation of reactive oxygen species (ROS) and down-regulated the expression of NADPH oxidase 1 (Nox1).

Pharmacokinetics

Amano H, Kazamori D, Itoh K. 2016. Xenobiotica. 46(11):1017-25.

S-methyl-L-cysteine (SMC) and trans-*S*-1-propenyl-L-cysteine (S1PC) given to models (2-5 mg/kg) were well absorbed with high bioavailability (88-100%). SMC and S1PC were excreted only to a small extent in the urine of models. The small renal clearance values (<0.03 l/h/kg) indicated the extensive renal reabsorption of SMC and S1PC, which potentially contributed to their long elimination half lives (>5 h) in models. However, the extent of undergoing the N-acetylation metabolism was extremely different between SMC and S1PC.

Amano H, Kazamori D, Itoh K. 2016. Biol Pharm Bull. 39(10):1701-7.

The effects of the three major organosulfur compounds in AGE, *S*-allyl-L-cysteine (SAC), *S*-methyl-L-cysteine (SMC), and trans-*S*-1-propenyl-L-cysteine (S1PC) were evaluated for their effects, and their N-acetylated and S-oxidized metabolites on five major isoforms of human CYP enzymes: CYP1A2, 2C9, 2C19, 2D6, and 3A4 using human liver microsomes. Results indicate that SAC, SMC and S1PC have little potential to cause drug-drug interaction due to CYP inhibition or activation *in vivo*, as judged by their minimal effects (IC_{50}>1 mM) on the activities of five major isofroms of human CYP *in vitro*.

Review

Nakamoto M, Kunimura K, et al. 2020. Exp Ther Med. 19(2):1550-3.

Garlic contains several hydrophobic antimicrobial compounds, such as vinyldithiins, ajoenes and diallyl polysulfides. Vinyldithiins are known to have several biological activities; however, they have no antimicrobial activity. Ajoenes (*Z*-ajoene and *E*-ajoene) were examined for antimicrobial activity against Gram-positive and –negative bacteria. The MIC values were 5-20 μg/ml for Gram-positives and 100-160 μg/ml for Gram-negatives, which *Z*-ajoene having slightly greater activity than *E*-ajoene. Both forms showed similar antimicrobial activity against 3 strains of *H. pylori* and are also active against fungi, such as *Aspergillus niger* and *Candida albicans*, but these compounds rapidly disappear after being mixed with blood. Diallyl polysulfides have limited antimicrobial activity against Gram-positive bacteria, including drug-resistant bacteria, and depend on the number of sulfur atoms in the molecules and are in order of diallyl tetrasulfide (DAS_4)>diallyl trisulfide (DAS_3)>diallyl disulfide (DAS_2)>diallyl sulfide (DAS_1).

Suzuki JI, Miki S, et al. 2020. Exp Ther Med. 19(2):1570-3.

This review summarizes the mechanisms through which the activation of autophagy by *S*-1-propenylcysteine (S1PC), a characteristic sulfur compound in AGE, modulates the immune response.

Kodera Y, Kurita M, et al. 2020. Exp Ther Med. 19(2):1574-84.

Three γ-glutamyl tripeptides [γ-glutamyl-γ-glutamyl-S-methylcysteine, γ-glutamyl-γ-glutamyl-S-allylcysteine (GGSAC), γ-glutamyl-γ-glutamyl-S-1-propenylcysteine], γ-glutamyl-S-allylmercaptocysteine (GSAMC) and cis-S-1-propenylcysteine (cis-S1PC) were isolated and

identified. A number of other compounds were also identified, including Maillard reaction products; however, their production mechanisms have not been elucidated. In this review, we present the changes in characteristic constituents in raw garlic and garlic extract during the aging process and discuss their production mechanisms involving the various chemical and enzymatic reactions.

Organosulfur Compounds in Aged Garlic Extract	
γ-glutamyl *S*-allyl cysteine	Diallyl trisulfide (DATS)
γ-glutamyl *S*-allyl mercaptocysteine	Vinyl-dithines
Ajoene	*S*-1-propenylcysteine (SPC)
Alliin	*S*-allyl cysteine (SAC)
Allyl methyl sulfide	*S*-methyl cysteine (SMC)
Cycloalliin	*S*-allyl mercaptocysteine (SAMC)
Diallyl disulfide (DADS)	*S*-1-propenyl-L-cysteine (S1PC)
Diallyl sulfide (DAS)	

Maillard Reaction Products

N-Fructosyl Glutamate and *Nα*-Fructosyl Arginine (Fru-Arg)

O'Brien J, Gillies DG. 1998. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. Nov 15-17, p. 66.

Ryu K, Ide N, et al. 2001. J Nutr. 131(3 Suppl):972S-6S.

Ryu K, Ide N, et al. 2001. Joint ACS-IcoFF Symposiium on Food Factors for Health Promotion. San Diego, CA. Apr 1-5. Abst #AGFD 51.

Two research teams lead by O'Brien et al. and Ryu et al. reported unique Maillard reaction products in AGE. These include *N*-fructosyl glutamate and *N*α-fructosyl arginine (Fru-Arg), which have antioxidant activity. They were generated through a non-enzymatic reaction of amino acids and reducing sugars during the aging of garlic. Fru-Arg completely scavenged hydrogen peroxide, a potent oxidant, at a very low dosage of 50 μM. Since Fru-Arg was detected in high levels in AGE, but not in raw or boiled garlic, its presence may partly explain the antioxidative effects of AGE not shown by other forms of garlic.

Ide N, Lau BHS, et al. 1999. J Nutr Biochem. 10(6):372-6.

Ide N, Ryu K, et al. 2001. 7th International Symposium on the Maillard Reaction. Kumamoto, Japan. Oct 29-Nov 1, p. 18.

*N*α-fructosyl arginine (Fru-Arg) was found to significantly inhibit the oxidizing effects of the copper ion (Cu^{2+}) on low-density lipoprotein (LDL) cholesterol when incubated together as shown by a reduction in thiobarbituric acid reactive substances (TBA-RS) formation. Pretreatment of pulmonary artery endothelial cells with Fru-Arg inhibited cell damage caused by oxidized LDL as indicated by a reduction in lactate dehydrogenase (LDH) release, an indicator of cell damage, and a reduction in TBA-RS formation. Incubation of Fru-Arg with macrophage immune cells also dose-dependently reduced the ability of oxidized LDL to initiate peroxide release from the macrophages. In a cell-free test tube system, Fru-Arg was shown to scavenge the hydrogen peroxide free radical.

Zhou H, Qu Z, et al. 2014. PloS One. 9(11):e113531.

AGE and N-α-(1-deoxy-D-frucos-1-yl)-L-arginine (FruArg) both were shown to significantly inhibit lipopolysaccharide (LPS)-induced nitric oxide (NO) production in in murine BV-2 microglial cells. Expressions of 26 proteins were significantly altered upon LPS exposure, while levels of 20 and 21 proteins exhibited significant changes in response to AGE and FruArg treatments, respectively, in LPS-stimulated BV-2 cells. Notably, approximately 78% of the proteins responding to AGE and FruArg treatments are in common. These results suggest that AGE and FruArg attenuate neuroinflammatory responses and promote resilience in LPS-activated BV-2 cells by suppressing NO production and by regulating expression of multiple protein targets with oxidative stress.

Song H, Lu Y, et al. 2016. Sci Rep. 6:35323.

Both AGE and N-α-(1-deoxy-D-fructos-1-yl)-L-arginine (FruArg) significantly attenuate lipopolysaccharide (LPS)-induced neuroinflammatory responses in BV-2 microglial cells. AGE reversed 67% of the mRNA alteration induced by LPS, whereas FruArg accounted for the protective effect by reversing expression levels of 55% of genes altered by LPS. Key pro-inflammatory canonical pathways induced by the LPS stimulation were modulated by treatment with both AGE and FruArg.

Other Maillard-type Products

Wakamatsu J, Stark TD, et al. 2019.

When AGE was heated at 100°C for 1 day, 12 new *in vitro* antioxidative Maillard-type products, α-[(2-

J Agric Food Chem. 67(8):2190-2200.

formyl-5-hydroxymethyl)pyrrol-1-yl]arginine (3), 4-[7-hydroxy-6-(hydroxymethyl)-7,8-dihydro-6 H-pyrano[2,3- b] pyrazine-3-yl]butane-1,2,3-triol (4), 4-[6-(1,2-dihydroxyethyl)-6,7-dihydro-furo[2,3-b]pyrazin-3-yl]-butane-1,2,3-triol (5), α-[(2-formyl-5-hydroxymethyl)-pyrrol-1-yl] aspartic acid (12), 1-[5-(1,2-dihydroxyethyl)-2-oxotetrahydrofuran-3-yl]-5-(hydroxymethyl)-1 H-pyrrole-2-carbaldehyde (14), 4-(6-ethyl-2-pyrazinyl)-1,2,3-butanetriol (17), α-[(2-formyl-5-hydroxymethyl)pyrrol-1-yl] glutamic acid (19), (S)-1-[(5-hydroxymethyl)furan-2-yl]methyl]-5-oxopyrrolidine-2-carboxylic acid (20), 3-hydroxy-1 H-[{5-(hydroxymethyl)furan-2-yl}methyl]-2,5-dioxo-3-pyrrolidine acetic acid (21), (E)-4-(5-methylpyrazin-2-yl)but-3-ene-7,2-diol (23), 4-acetyl-6-(hydroxymethyl)picolinic acid (24), (E)-4-(6-methylpyrazin-2-yl)but-3-ene-1,2-diol (26) and 14 known compounds, 1, 2, 6-11, 13, 15, 16, 18, 22 and 25, which were characterized via 1D/2D-NMR, CD spectroscopy, and mass spectrometry. ARS and ORAC activities of these antioxidants ranged from 0.01 to 0.49 µmol TE/µmol and from 0.01 to 3.50 µmol TE/µmol, respectively. Additionally, plausible formation pathways for the new organic acid-type products (15, 20, and 21) were proposed based on proving their generation in model reactions detected via liquid chromatography-mass spectrometry (LC-MS/MS).

Tetrahydro-β-carboline Derivatives

Ide N, Ichikawa M, et al. 2001. Joint ACS-IcoFF Symposium on Food Factors for Health Promotion. San Diego, CA. Apr 1-5. Abst #AGFD 32.

Ide N, Ryu K, et al. 2001. 7th International Symposium on the Maillard Reaction. Kumamoto, Japan. Oct 29-Nov 1, p. 18.

Ide N, Ichikawa M, et al. 2002. In: International Congress Series 1245. The Maillard Reaction in Food Chemistry and Medical Science: Update for the Postgenomic Era. Elsevier Science, B.V., pp. 449-50.

Ide et al. reported that they found four of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids (MTCCs) as antioxidants in AGE. Interestingly, the concentrations of these compounds in AGE were shown to increase in progression of the natural aging process. Antioxidant properties of these compounds were further studied using several *in vitro* assay systems. All of four MTCCs showed strong hydrogen peroxide activities, particularly (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid [(1S, 3S)-MTCdiC], which was the most potent hydrogen peroxide scavenger and the activity was stronger than ascorbic acid. To elucidate the mechanism, data suggests that the metabolite of (1S, 3S)-MTCdiC may function as an electron donor and scavenge hydrogen peroxide. MTCCs also inhibited the peroxidation of linoleic acid caused by incubating with 2,2'-azobis(2-amidinopropane) hydrochloride [AAPH] at 37°C. Macrophages were incubated with lipopolysaccharides (LPS) at 37°C and 5% CO_2 for 20 hours and the release of nitric oxide (NO) metabolites were measured using a spectrophotometer. LPS significantly increased the release of NO metabolites from macrophages. Among four MTCCs identified in AGE, only dicarboxylates (1S, 3S) and (1R, 3S)-MTCdiC, significantly inhibited the release at low concentrations, suggesting that MTCCs, which are formed during the natural aging process, are potent antioxidants in AGE and that AGE would be useful for prevention of disorders associated with oxidative stress.

Yoshida J, Ide N, et al. 2001. 6th Annual Meeting of Japanese Society of Food Factors (JsoFF). Kobe, Japan. Nov 29-30, p. 22.

Four new antioxidants identified in AGE were reported. These compounds, 1,2,3,4-tetrahydro-β – carboline derivatives, showed strong scavenging activities. Among these compounds, (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline—1,3-dicarboxylic acid was found to be stronger than ascorbic acid. Chemical analytical data indicates that these four compounds were not detected in raw garlic, but their presence was increased during the natural aging process. These four new compounds may contribute to the antioxidant activities of AGE.

Ichikawa M, Ryu K, et al .2002. BioFactors. 16(3-4):57-72.

Ichikawa M, Ryu K, et al. 2004. Ch. 28. In: ACS Series 871. Nutraceutical Beverages: Chemistry, Nutrition and Health Effects, pp. 380-440.

Ichikawa M, Yoshida J, et al. 2006. J Nutr. 136(3 Suppl):726S-31S.

In a study that used liquid chromatography mass spectrometry (LC-MS), four tetrahydro-β-carboline derivatives were found to have strong hydrogen peroxide scavenging activities. These compounds found in AGE were shown to increase during the aging process and were not detected in raw garlic. This study suggests that these compounds are potent antioxidants and may play an important role in preventing disorders that are associated with oxidative stress.

Wang X, Liu R, et al. 2015. Food Chem. 187:37-43.

The major antioxidants: l-phenylalanine, l-tryptophan, (3S)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, and (1R,3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, were isolated, purified and identified in AGE.

Steroidal Glycosides

Marker Compounds for Garlic

Matsuura H. 2001. J Nutr. 131(3 Suppl):1000S-5S.

Matsuura et al. showed that garlic cloves contain a furostanol saponin proto-eruboside-B. When garlic cloves are processed, a glucose molecule is removed from proto-eruboside-B through an enzymatic hydrolysis releasing eruboside B. Eruboside B consists of four sugar moieties (one glucose and three galactose) and a unique steroid aglycone, named β-chlorogenin.

Itakura Y, Ichikawa M, et al. 2001. J Nutr. 131(3 Suppl):963S-7S.

After analyzing various saponin profiles in the *Allium* species, β-chlorogenin could be the best candidate for identification and distinction of garlic from the other *Allium* vegetables.

Showed Cardioprotective Activity

Matsuura H, Slowing K, et al. 2000. Phytomedicine. 7(2):48. Abst #SL-98.

Matsuura H. 2001. J Nutr. 131(3 Suppl):1000S-5S.

The steroid saponins in garlic were isolated and the structures were determined, which exhibit cholesterol-lowering effects. Elevated cholesterol levels were produced in models by feeding them a cholesterol-enriched diet for 16 weeks. A significant reduction in total plasma cholesterol was found in models ingesting the crude glycoside fraction. It was suggested that steroid saponins should be considered active compounds responsible for the cholesterol-lowering effects of garlic and its preparations.

Slowing K, Ganado P, et al. 2001. J Nutr. 131(3 Suppl):994S-9S.

Slowing et al. showed research that indicated that the saponin fraction of garlic reduced serum cholesterol levels and prevented loss of vascular reactivity in models fed with a cholesterol-enriched diet.

Showed Anti-Fungal Activity

Matsuura H, Ushiroguchi T, et al. 1988. Chem Pharm Bull. 36(9):3659-63.

Matsuura H, Ushiroguchi T, et al. 1989. Chem Pharm Bull. 37(10):2741-3.

New steroidal glycosides from garlic were identified and isolated, which belong to the saponin group and have anti-fungal activity.

F-4 Protein Fraction

Enhanced Immune Cell Activity/Anti-Tumor Effects

Hirao Y, Sumioka I, et al. 1987. Phytother Res. 1:161-4.

The F-4 protein fraction in AGE strongly stimulated peritoneal macrophage activity and exhibited cytostatic activity *in vitro*. F-4 also stimulated the proliferating activity of spleen vells. *In vivo*, F-4 induced the stimulation of carbon clearance activity. F-4 was concluded to be effective for the suppression of tumor cell outgrowth through the stimulation of immunoresponder cells.

Lau BHS, Yamasaki T, et al. 1991. Mol Biother. 3(2):103-7.

F-4 protein fraction from AGE was found to stimulate the proliferation of T-lymphocytes.

Morioka N, Sze LL, et al. 1993. Cancer Immunol Immunother. 37(5):316-22.

A protein fraction (F-4) isolated from AGE was found to enhance the ability of human peripheral blood lymphocytes to destroy tumor cells. Moreover, F-4 significantly stimulated the lymphokine interleukin-2 (IL-2)-activated killer activity. F-4 also enhanced the proliferation of lymphocytes induced by the immune-stimulating agents interleukin-2 and concanavalin-A, suggesting a possible reduction of the dosage of interleukin-2 in cancer immunotherapy.

Enhanced Nerve Survival

Nishiyama N, Moriguchi T, et al. 1996. Int Acad Biomed Drug Res. Basel Karger. 11:253-8.

Moriguchi T, Nishiyama N, et al. 1996. Phytother Res. 10:468-72.

AGE's F-4 protein fraction markedly increased the survival of cultured hippocampal neurons, the first clear evidence that AGE interacts with brain neurons.

Enhanced Growth of Friendly Bacteria *B. bifidum* and *L. acidophilus*

Matsuura H. 1997. Ch. 7. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Lachance PP (ed). Food & Nutrition Press. Trumbell, CT, pp. 55-69.

Kokai Tokyo Koho, Japanese Patent H1-252276.

The F-4 protein fraction in AGE enhanced the growth of the beneficial bacteria *Bifidobacterium bidifum* and *Lactobacillus acidophilus*. There is an international patent (#H1-252276) for this effect.

Allixin

Anti-Microbial Activity

Kodera Y, Matsuura H, et al. 1989. Chem Pharm Bull. 37(6):1656-8.

Kodera et al. isolated a phenolic stress compound from garlic and termed it allixin. Allixin was found to possess a weak antimicrobial activity.

Kodera Y, Ayabe M, et al. 2002. Chem Pharm Bull. 50(3):405-7.

Allixin, a *de novo* synthesized substance categorized as a phytoalexin, may pose a prohibitory, inhibitory or post-inhibitory antimicrobial function in garlic. The basis of this conclusion comes from the observation of high accumulation of allixin in nectrotic tissue areas after long-term storage.

In vivo Anti-Tumor Activity

Nishino H, Nishino A, et al. 1990. Cancer J. 3(1):20-1.

Allixin demonstrated in vitro anti-tumor activity and suppressed the promotion of two-stage carcinogenesis *in vivo*. Allixin inhibited the development of skin cancer induced by the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) and the promoter 12-O-tetradecanoyl (TPA). It was suggested that since allixin seems to produce no side effects, it may be useful for the prevention of human cancer.

Antimutagenic and Chemopreventive Activity

Yamasaki T, Teel RW, et al. 1991. Cancer Lett. 59(2):89-94.

Allixin showed a dose-related inhibition of histidine^{+} revertants induced by aflatoxin B_1 (AFB_1). Allixin prevented the binding of this carcinogen to calf thymus DNA and reduced the formation of AFB_1-DNA adducts. Allixin also inhibited the formation of carcinogenic metabolites of this aflatoxin and therefore, it was suggested that this compound may be useful in the chemoprevention of cancer.

Enhanced Nerve Survival

Nishiyama N, Moriguchi T, et al. 1996. 116th Annual Meeting of the Pharmaceutical Society of Japan. Kanazawa, Japan. Mar 27-29. Abst #27(J1)10-2.

Nishiyama et al. reported the effect of allixin, one of the stress compounds in garlic, on survival of neuron cells using a cell culture system. The neuron cells cannot survive without serum and began to die in 72 hours. Neuron cells were coincubated with allixin for 48 hours and the survival rate and condition of the cells were examined. Results indicate incubation with allixin demonstrated a significant improvement in survival rate in a dose-dependent manner and promoted the branching of cells. In order to reduce the side effects, garlic's derivatives were chemically synthesized and the survival rate of nerve cells was studied. As a result, 2,6-dimethyl-3-hydroxy-4-pyrone (DHP) was found to show similar activity with less toxicity. These two compounds may be useful as external neurotrophics.

Chemistry

Kodera Y, Ayabe M, et al. 2001. Chem Pharm Bull. 49(12):1636-7.

Allixin was induced by irradiating fresh garlic cloves with sunlight or UV light. The induced allixin wsa analyzed by high-performance liquid chromatography (HPLC) and the accumulated amounts of allixin were 3.1 to 6.3 μg/g under these conditions. The importance of this study presents the possibility of allixin induction by light irradiation.

Selenium

Koch HP, Jager W, et al. 1988. Dtsch Apoth Ztg. 128(19):993-5.

In a comparative study among various garlic products on the market in Germany, Kyolic AGE was found to have the most selenium of the eight products tested, containing 25-50% the selenium of raw garlic.

El-Bayoumy K, Raghu S, et al. 2006. J Nutr. 136(3 Suppl):864S-9S.

Selenium compounds in garlic exhibited chemopreventive effects by inhibiting the development of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary adenocarcinomas and azoxymethane-induced colon cancer and benzo[a]pyrene-induced forestomach tumors.

Tsuneyoshi T, Yoshida J, et al. 2006. J Nutr. 136(3 Suppl):870S-2S.

Using high-performance liquid chromatography inductively coupled plasma MS (HPLC-ICP-MS) analysis, Selenium (*Se)*-methlyselenocysteine (MeSeCys), γ-glutamyl-*Se*-methlyselenocysteine (γ-GluSeMeCys), selenomethionine and nonmetabolized selenate were identified in water extracts of garlic seedlings when hydroponic enrichment of *Se* was implemented. High *Se* garlic could be an ideal nutritional supplement of dietary *Se* for cancer prevention.

Peroxidase

Kitahara S, Takahara S, et al. 1974. Kiso to Rinsho (Preclin Clin Rep). 8(12):115-6.

Peroxidase, which decomposes the oxidizing compound hydrogen peroxide which is produced by vital reaction, is found in many vegetables and plants, including garlic. The degree of peroxidase activity in garlic products varies substantially. Kyoleopin® (KLE) and Leopin-5® (LE-5) were found to have peroxidase activity. Studies of five other garlic products on the market showed no such activity.

Others

Ichikawa M, Ryu K, et al. 2003. J Agric Food Chem. 51(25):7313-7.

New antioxidant compounds were found in garlic skin. These compounds are also found in AGE. Six phenylpropanoids were identified. Determination and assay of these chemical compounds have been done by state-of-the-art chemical analysis such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), etc.

Chandrashekar PM, Venkatesh YP. 2009. J Ethnopharmacol. 124(3):384-90.

Changes occurring in garlic during aging were examined and two immunomodulatory proteins found in AGE were identified. The purified protein components QA-1, QA-2 and QA-3 display immunomodulatory and mannose-binding activity; QA-2 shows the highest mitogenic activity. The identity of QA-2 and QA-1 proteins with the garlic lectins ASA I and ASA II, respectively, has been confirmed by hematgglutination analysis. QA-3 exhibits mitogenic activity but not hemagglutination activity.

Chandrashekar PM, Prashanth KVH, et al. 2011. Phytochemistry. 72(2-3):255-64.

In addition to organosulfur compounds, AGE also contains fructans, which the amount represents a small fraction (0.22%) of the total fructans in raw garlic. Both high molecular weight (HF) and low molecular weight (LF) fructans were isolated from AGE and have (2 → 1) β-D-fructofuranosyl bonds linked to a terminal glucose at the non-reducing end and β-D-fructofuranosyl branching on its backbone. Both HF and LF displayed mitogenic activity and activation of macrophages including phagocytosis. These activities were comparable to that of known polysaccharide immunomodulators such as zymosan and mannan. This study clearly demonstrates that garlic fructans also contribute to the immunomodulatory properties of AGE, and is the first such study on the biological effects of garlic fructans.

Yamakawa T, Matsutomo T, et al. 2014. Food Nutr Sci. 5(4):177-84.

It was found that primary human coronary artery smooth muscle cells (HCASMC) showed the increase alkaline phosphatase (ALP) activity, when cultured with ascorbic acid, β-glycerophosphate, dexamethasone (IM), and supplemented with conditioned medium from macrophages (MCM). Then the effect of AGE subdivided fractions and several compounds found in AGE was tested, and it was found that ((+)-(2S,3R)-dehydrodiconiferyl alcohol, a dilignol compound existed in hydrophobic fraction of AGE, inhibited ALP activity in HCASMC.

Tsuneyoshi T, Kanamori Y, et al. 2015. Biochem Biophys Res Commun. 465(3):408-13.

Antioxidant lignan compounds isolated from AGE (+)-(2S,3R)-dehydroconiferyl alcohol (DDC) and (-)-(2R,3S)-dihydroehydroiconiferyl alcohol (DDDC) were shown to suppress the adhesion of THP-1 monocyte to human umbilical vein endothelial cells (HUVECs) which was activated by

lipopolysaccharide (LPS) or advanced glycation endproducts (AGEs-BSA) and inhibited vascular cell adhesion molecule 1 (VCAM-1) expression induced by LPS or AGEs-BSA, although DDDC was less effective than DDC. In addition, the inhibitory effect of DDC on VCAM-1 expression involved suppressing JNK/c-Jun pathway. These results suggest that DDC may improve endothelial dysfunction.

Park JB. 2016. J Nutr. 146(2):437S-443S.

Alfrutamide and caffedymine are phenolic amides found in garlic, are studied for bioavailability and their effects on P-selectin expression (PSE) and platelet-leukocyte aggregation (PLA), which are frequently involved in the progression of cardiovascular diseases (CVDs), using models. At 0.05 μmol/L, alfrutamide and caffedymine inhibited cyclooxygenase (COX) I and COX-II activities by 20-40% ($P < 0.05$) and 16-23% ($P < 0.05$), respectively, compared with control. At 0.1 μmol/L, alfrutamide and caffedymine also inhibited platelet PSE by 28% ($P < 0.05$) and 35% ($P < 0.05$), respectively, compared with control. The β2-adrenoceptor antagonists ICI118551 and butoxamine partially suppressed the inhibition of PSE by caffedymine. At the same concentration, these 2 compounds inhibited PLA by 24-32% ($P < 0.05$) compared with the control. In addition, caffedymine and alfrutamide administered orally to models exhibited maximum concentrations >0.6 μmol/L and significant inhibition of PSE by 23-34% ($P < 0.05$) and PLA by 20-27% ($P < 0.05$) compared with control models.

Abe K, Hori Y, et al. 2020. Exp Ther Med. 19(2):1585-93.

This review summarizes the volatile components of fresh and processed garlic, particularly those produced by heating and aging. The pungent odor of fresh garlic is contributed mainly to thiosulfinates and their degradation products. During the heating process of garlic, thiosulfinates are mainly decomposed, and nitrogen-containing volatile compounds, such as pyridines and pyrazines are generated. Aldehydes are dominant compounds in black garlic, while esters and phenols are key aroma compounds in AGE. The slight variations in chemical reactions during the aging process may lead to differences in the aroma of the two types of garlic.

CHEMISTRY OF AGED GARLIC EXTRACT

Matsuura H. 2000. 6th Annual Meeting of Jpn Mibyou System Assoc. Hiroshima, Japan. Jan 28, p. 41.

The history of garlic use in folk medicine dates back in ancient times when it was supplied to the workers building the pyramids. Even the ancient Roman civilization used garlic as a tonic. Currently, garlic oil is one of the most studied plants with most research focusing on its unique chemistry, which contains more than 5% sulfur compounds and its biological activities, such as cholesterol lowering effects and anti-platelet aggregation. Thus, the focus of this paper is the history of garlic and research completed on its unique chemistry.

Ide N, Ichikawa M, et al. 2001. Joint ACS-IcoFF Symposium on Food Factors for Health Promotion. San Diego, CA. Apr 1-5. Abst #AGFD 32.

Ide N, Ichikawa M, et al. 2002. In: International Congress Series 1245. The Maillard Reaction in Food Chemistry and Medical Science: Update for The Postgenomic Era. Elsevier Science B.V., pp. 449-50.

Using liquid chromatography-mass spectrometry (LC-MS), the antioxidant effects of four tetrahydro-β-carboline derivatives were determined. These compounds were found to increasing during the aging process and may be an important antioxidant in AGE.

Kodera Y, Matsuura H, et al. 2001. Joint ACS-IcoFF Symposium on Food Factors for Health Promotion. San Diego, CA. Apr 1-5. Abst #AGFD 108.

The biological activities of water-soluble compounds derived from garlic such as *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC), have become the center of attention because they are odorless and safe. *S*-allyl groups play a significant role in the pharmacological activities of organosulfur compounds derived from garlic based on studies on structure-activity relationships.

Ide N, Ryu K, et al. 2002. In: International Congress Series 1245. The Maillard Reaction in Food Chemistry and Medical Science: Update for The Postgenomic Era. Elsevier Science B.V., pp. 447-8.

Ide et al. used several *in vitro* assay systems and high performance liquid chromatography (HPLC) to determine the antioxidant effects of fructosyl arginine (Fru-Arg), a compound in AGE. The study reported that Fru-Arg forms and is increased during the aging process and plays an important role as an antioxidant.

Ryu K, Matsuo H, et al. 2002. 223rd ACS National Meeting. Orlando, FL. Apr 7-11. Abst #AGFD 104.

In a review on AGE, it was stated how garlic gains antioxidant activity during the aging process via different constituents found in garlic: *S*-allylcysteine (SAC), fructosyl-arginine (Fru-Arg) and tetrahydro-β-carbolines. Liquid chromatography mass spectrometry (LC/MS) data showed all of the compounds were either not present or were in extremely lower concentrations in raw garlic, but were increased during the

aging process.

Kodera Y, Matsuura H, et al. 2003. Ch. 30. In: ACS Series 851. Food Factors in Health Promotion and Disease Prevention. American Chemical Society, pp. 346-57.

The unique characteristics of AGE and the cascade of chemical reactions of garlic are explained in this article. Many unique chemical constituents in AGE were listed and pharmacokinetic behavior *in vivo* is reviewed, which is essential to the true active compounds.

Thomson M, Ali M. 2003. Curr Cancer Drug Targets. 3(1):67-81.

S-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC), present in AGE, were found to have the highest activity of scavenging free radicals. SAC, in several disease models, was also shown to decrease the growth of both chemically induced and transplantable tumors. This review suggests that garlic consumption may play an important role in the protection from the development of cancer.

Ryu K, Ide N, et al. 2003. Ch. 23. In: ACS Series 871. Food Factors in Health Promotion and Disease Prevention. American Chemical Society, pp. 264-73.

Fructosyl-arginine (Fru-Arg) is a unique compound only found in AGE and no other garlic products have this kind of compounds in it. Fru-Arg has a strong anti-oxidant effect and state-of-the-art analytical technology with sensitive liquid chromatography-mass spectrometry (LC-MS) method has been developed and reported for this unique compound.

Amagase H, Rosen RT. 2004. International Congress on Natural Products Research. Jul 31-Aug 4.

Allicin is not active nor a marker compound in garlic products. AGE uses *S*-allyl cysteine (SAC) since it is bioavailable and active in the body. It is reasonable to use such compound for standardization.

Ichikawa M, Ide N, et al. 2006. J Agric Food Chem. 54(13):4849-54.

Ichikawa et al. studied the changes in organosulfur compounds in garlic cloves during storage at different temperatures. Results indicated that γ-glutamyl peptides undergo marked conversion to sulfoxides when garlic cloves are stored at 4°C. They also demonstrated that isoalliin produced enzymatically from γ-L-glutamyl-*S*-(trans-1-propenyl)-L-cysteine (GSPC) is chemically converted to cycloalliin and that the cycloalliin content increases when garlic cloves are stored at higher temperatures.

Ichikawa M, Mizuno I, et al. 2006. J Agric Food Chem. 54(26):9811-9.

Although less bioavailable (<10%) than *S*-allyl cysteine (SAC) in garlic, cycloalliin is more suitable as a chemical marker for garlic due to its highly stable nature when stored or processed.

Kodera Y, Ichikawa M, et al. 2007. International Conference on Food Factors for Health Promotion (IcoFF). Kyoto, Japan. Nov 27-Dec 1. Abst #S22-3.

Numerous compounds derived from the *Allium* species have been reported, however, the characteristic property of these compounds is the presence of a sulfur molecule, which is believed to have an important role in biological activities. Recently, biological activities of water-soluble organosulfur compounds derived from garlic have received much attention in garlic preparations because they are odorless, safe and stable. Therefore, the pharmacokinetics properties of water-soluble organosulfur compound needs to be evaluated to understand benefit of phytochemicals in *Allium* plants for our health. This report focuses on the pharmacokinetic property of cysteine derivatives from garlic.

Cope K, Seifried H, et al. 2009. Anal Biochem. 394(2):243-8.

A new method for extraction and sensitive detection of both *N*-nitrosoproline (NPRO) and *N*-acetyl-*S*-allylcysteine from urine was presented. NPRO excretion has been used as an index for endogenous nitrosation. Urine samples from a study were analyzed to test whether garlic supplementation inhibits NPRO synthesis. Using the method, NPRO and *N*-acetyl-*S*-allylcysteine were quantified and detected in urine. The results suggest that 3 to 5 g of garlic supplements inhibited NPRO synthesis to an extent similar to a 0.5g dose of ascorbic acid or a commercial supplement of AGE. Urinary NPRO concentration was inversely associated with the *N*-acetyl-*S*-allylcysteine concentration.

Nakabayashi R, Sawada Y, et al. 2016. J Nutr. 146(2):397S-402S.

Sulfur-containing metabolites were detected and chemically assigned in this study using LC—Fourier transform ion cyclotron resonance—mass spectrometry (FTICR-MS) in *Allium* plants, including garlic. Putative 69 S-containing monoisotopic ions (S-ions) were extracted from the metabolome data of onion, green onion and garlic (*Allium sativum*). Eight S-ions were chemically assigned by using the reference data according to the guidelines of the Metabolomics Standards Initiative. Three ions detected in garlic were assigned as derived from the isomers γ-glutamyl-*S*-1-propenylcysteine and γ-glutamyl-*S*-2-propenylcysteine and as *S*-2-propenylmercaptoglutathione on the basis of differences in key product ions identified in reference tandem MS spectra.

Matsumoto T, Kodera Y. 2016. J Nutr. 146(2):450S-455S.

A rapid postcolumn HPLC method for both qualitative and quantitative analyses of sulfur compounds was developed and this method helped elucidate a potential mechanism of *cis*-*S*-1-propenylcysteine (*cis*-S1PC) and *S*-allylmercaptocysteine (SAMC) action in AGE. The advantages of this new method includes less interference from nonsulfur compounds, high sensitivity, good correlation coefficients (r>0.98), and high resolution that can separate >20 sulfur compounds, including several isomers, in garlic preparations in a single run.

Martins N, Petropoulos S, et al. 2016. Food Chem. 211:41-50.

This review examines all aspects related with garlic chemical composition and quality, focusing in its bioactive properties. The organosulfur compounds content is emphasized since they highly contribute to the effective bioactive properties of garlic, including its derived products. Also discussed are pre-harvest (genotype and various cultivation practices) and post-harvest conditions (storage conditions and processing treatments) on chemical composition and, consequently, bioactive potency of garlic.

Fujii T, Matsutomo T, et al. 2018. J Agric Food Chem. 66(40:10506-12.

γ-Glutamyl-*S*-allylmercaptocysteine (GSAMC), a putative precursor compounds of *S*-allylmercaptocysteine (SAMC), was isolated and identified from AGE. The change of their contents in AGE during the aging process was analyzed chronologically 1 month to 22 months. SAMC content reached a maximum at approximately 4 months, whereas GSAMC content reached a maximum at 1 month and then decreased during the subsequent aging period. Using model reactions, SAMC was produced from GSAMC by the garlic protein fraction having γ-glutamyl transpeptidase (GGT) activity, and its production was suppressed by a GGT inhibitor. Furthermore, the production of GSAMC from allicin and γ-glutamyl-*S*-allycysteine (GSAC)/γ-glutamyl-*S*-1-propenylcysteine (GS1PC) was found in another model reaction. The reaction between allicin and GS1PC was faster than that between allicin and GSAC, and thus may be involved in the production of GSAMC in the early stage of aging process.

Nakamoto M, Fujii T, et al. 2018. J Agric Food Chem. 66(11):2891-9.

Three γ-glutamyl tripeptides were isolated from AGE and identified as γ-glutamyl-γ-glutamyl- *S*-methylcysteine, γ-glutamyl-γ-glutamyl- *S*-allylcysteine (GGSAC) and γ-glutamyl-γ-glutamyl- *S*-1-propenyl-cysteine (GGS1PC). GGSAC and GGS1PC were novel compounds. Trace amounts of these compounds were detected in raw garlic, but the contents of these compounds are increased during the aging process. Production of these compounds was inhibited using a γ-glutamyl transpeptidase (GGT) inhibitor in the model reaction mixtures.

REVIEW OF THE BENEFITS OF AGE, SAC AND SAMC

Rivlin RS. 2001. J Nutr. 131(3 Suppl):951S-4S.

Pinto JT, Krasnikov BF, et al. 2006. J Nutr. 136(3 Suppl):835S-41S.

The sulfur-containing compounds in garlic may modify cancer-cell growth by targeting antioxidant pathway, which regulates cell growth or death. Among the various chemicals, *S*-allyl mercaptocysteine (SAMC), only found in AGE, has significant effect on this pathway. Detailed cancer cell control by SAMC is described.

Milner JA. 2001. J Nutr. 131(3 Suppl):1027S-31S.

In a review on garlic, both water- and lipid-soluble allyl sulfur compounds are effective in blocking a myriad of chemically-induced tumors through different mechanisms and reactions that happen in the body. Also, the anticarcinogenic potential of garlic can be influenced by several dietary components including specific fatty acids, selenium and vitamin A.

Amagase H, Petesch B. 2003. In: Encyclopedia of Food Sciences and Nutrition. Elsevier Science, Ltd., pp. 2861-4.

Garlic, especially AGE, has been mentioned and reviewed in this article as part of the Encyclopedia of Food Sciences ad Nutrition. It introduces a wide variety of benefits of garlic based upon the scientific literature of AGE.

Ryu K, Rosen RT. 2003. Ch. 19. In: Oriental Foods and Herbs: Chemistry and Health Benefits. Lin JK, Ho CT, Zheng Qy (eds). Oxford University Press. New York City, NY, pp. 258-70.

In a study using high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS), *S*-allyl-L-cysteine (SAC), fructosylarginine and 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids were shown to be principal bioactive compounds of AGE. These three compounds were present and found to be more stable and bioactive than thiosulfinates. The levels of these components were also found to increase during the aging process. This suggests that additional health benefits may be provided by the aging process of garlic.

Borek C. 2004. Townsend Letter for Doctors & Patients. Aug/Sep, pp. 112-5.

In a review, Borek discussed the numerous benefits of AGE. With over 580 research studies, AGE has been shown to be beneficial in many areas including cardiovascular protection (i.e., lowering cholesterol, blood pressure, etc.), increase in circulation, enhance immunity and preventing many forms of cancer and neurodegenerative disease. AGE has also been shown to have anti-aging effects that may help with improving memory, endurance and learning.

Tapsell LC, Hemphill I, et al. 2006. Med J Aust. 185(4 Suppl):4S-24S.

The health benefits of culinary herbs and spices are discussed. In particular, a review of AGE studies indicates that it may prove beneficial in cardiovascular health. Clinical trials examine the effect of garlic on lipid levels and lipoproteins, as well as its anti-clotting and blood pressure effects. Other studies find that herbs and spices, including garlic and its constituents, may have an inhibitory effect on carcinogens at all stages of the cancer process by interfering with Phase I and Phase II enzymes.

Sener G, Sakarcan A, et al. 2007. Mol Nutr Food Res. 51(11):1345-52.

Water-soluble organosulfur compounds, *S*-allyl cysteine (SAC) and lipid-soluble compounds like diallyl sulfide (DAS) have shown antioxidant properties. The *in vivo* and *in vitro* ischemia reperfusion studies showed that prophylactic administration of aqueous garlic prior to ischemia reperfusion inhibit lipid peroxidation and prevent depletion in glutathione through its compounds that led to functional recovery. Its ability to inhibit neutrophil migration could suppress fibrosis formation. These preventive effects are seen in models that studied organs such as kidney and liver with functional recovery. Future studies should focus on post ischemia reperfusion administration of garlic to explore its rescue potential rather than prophylactic impact.

Mann J, Bernstein Y, et al. 2020. Exp Ther Med. 19(2):1504-6.

This review discusses gingivitis, periodontitis and peri-implantitis with a brief focus on the different primary prevention aids, such as mechanical, chemical and the new generation of potential products to be used in the future dental market, products that are prepared from natural sources, the general trend worldwide. The role of AGE is described for the prevention of periodontal disease.

Ohtani M, Nishimura T. 2020. Exp Ther Med. 19(2):1507-10.

To date, at least to the best of our knowledge, there is no clinical report available on the therapeutic effects of garlic or its extract on periodontal diseases, apart from a recent study, which reported that the intake of AGE containing various pharmacologically active sulfur compounds, alleviated symptoms of gingivitis clinically. The finding suggests that AGE may be a promising candidate for use in the treatment of periodontal diseases, although additional clinical trials are warranted to confirm this. In addition, further studies are required for the clarification of the basic molecular mechanisms through which AGE attenuates gingivitis. In this review, we summarize the beneficial effects of several natural compounds on periodontal disease and describe the possible applications of garlic ingredients in detail.

HISTORY

Rivlin RS. 2006. J Nutr. 136(3 Suppl):713S-5S.

The ancient tradition of utilizing garlic would qualify it as part of the “folk medicine” or “alternative/complementary medicine.” Scientists around the world have identified a number of bioactive substances in garlic that are water-soluble (e.g., *S*-allylmethylcysteine) found in AGE, and fat-soluble (e.g., *S*-allylsulfide). Mechanisms of action are being elucidated by modern technology. The current state of knowledge will find a place as a “complement” to known methods of disease prevention and treatment. Our goal now should be to examine garlic together with other agents to evaluate possible efficacy and toxicity under conditions of actual use in humans.

REGULATORY ISSUES

Kroes B. 2006. J Nutr. 136(3 Suppl):732S-5S.

Garlic products are marketed in the European Union (EU) as foodstuffs and herbal medicinal products. Although all EU countries have garlic foodstuffs in their markets, garlic medicinal products are available only in a limited number of EU member states due to discrepancies among national laws. This article reviews the status of garlic products in EU countries and their approved medical claims and discusses legislative options for the authorization of garlic products and medicinal products.

GLOSSARY

Aged Garlic Extract – Kyolic®; organically grown garlic aged with purified, filtered water and alcohol in stainless steel tanks for up to 20 months
Caco-2 – human epithelial colorectal adenocarcinoma cells/cell line
CAL-27 – human oral squamous cancer cells
CRL-1740 –human prostate carcinoma/cancer cells/cell line
de novo – from the beginning, afresh, anew (Latin)
DS19 – murine erythroleukemia cells
HCT-15/16 – human colon cancer/carcinoma/tumor cells
HEL – human erythroleukemia cells/cell line
HepG2 – human hepatocellular carcinoma cells/cell line
HT-29 – human colon cancer cells/cell line
IEC-6 – murine intestinal epithelial cells
in vitro – in glass (Latin)
in vivo – within the living (Latin)
in situ – in position (Latin)
LA-N-5 – human neuroblastoma cells/cell line
MHCC97L – hepatocellular carcinoma cells/cell line
Kyoleopin® – Aged Garlic Extract with vitamin B_1, vitamin B_{12} and liver extract (Japan)
Kyoleopin Neo® – Aged Garlic Extract with vitamin B_6, nicotinamide, panthenol and liver extract (Japan)
Kyolic® - Aged Garlic Extract (USA)
Leopin-5® –Aged Garlic Extract with vitamin B_1, Korean ginseng extract and bezoar bovis tincture (Japan)
Leopin Royal® –Aged Garlic Extract with ginseng, oriental bezoar, antler velvet, cuscuta seed and epimedium herb (Japan)
LL/2 – Lewis Lung Carcinoma cell line
LLC-PK_1 – renal epithelial cells
LNCaP – androgen-sensitive human prostate adenocarcinoma cells/cell line
MBT2 – murine bladder carcinoma cells/cell line
NSCLC – human non-small-cell lung carcinoma
OCIM-1 – human erythroleukemia cells/cell line
PC-3 – human prostate cancer cells
PC12 – pheochromocytoma of muroid adrenal medulla cells/cell line
RBL-2H – mast cell model
SK-N-SH – human neuroblastoma cells/cell line
SW480/620 – human colon cancer cells/cell line
TEV-1 – human first-trimester extravillous trophoblast cells/cell line
THP-1 – human acute monocytic leukemia cell line

LIST OF ABBREVIATIONS

3-NPA – 3-nitropropionic acid
4-HNE – 4-hydroxy-2-nonenal
5-FU – 5-fluorouracil
5-HT – 5-hydroxytryptamine or serotonin
6-OHCZX – 6-OH hydroxychlorzoxazone
8-iso-PGF2α – 8-iso-prostaglandin-2Fα
8-OhdG – 8-hydroxydeoxyguanosine/8-hydroxy-2-deoxyguanosine
α2MRP – alpha-2-microglobulin-related protein
α-SMA – alpha-smooth muscle actin
AA –arachidonic acid
AAPH – 2,2'-azo-bis(2-amidinopropane) HCl
Aβ/Abeta – amyloid-beta, beta-amyloid
Aβ40/Aβ42 – oligomer of Aβ/Abeta
ABCA1 – adenosine triphosphate-binding cassette transporter A1
ACF – aberrant crypt foci
ACTH – adrenocorticotropic hormone
AD – Alzheimer's disease
ADP – adenosine diphosphate
AFB_1 – aflatoxin B_1
AGE – Aged Garlic Extract
AGEP – advanced glycation endproduct
AIDS – acquired immunodeficiency syndrome
ALP – alkaline phosphatase
ALT – alanine aminotransferase
Amyloid-β – amyloid-beta, beta-amyloid or Abeta protein
AM – allyl mercaptan
AMD – allyl methyl disulfide
AMT – allyl methyl trisulfide
anti-SRBC – anti-sheep red blood cell
AOM – azoxymethane
AOSC – organosulfur compounds in alliums
APAP – acetaminophen
ApcI-MS – atmospheric-pressure chemical ionization-mass spectrometry
apoB – apolipoprotein B-100
APP – beta-amyloid precursor protein
AR – aldose reductase
AST – aspartate aminotransferase
ATMT – Advanced Trail Making Test
ATP – adenosine triphosphate
β-amyloid –beta-amyloid, amyloid-beta, Abeta
BACE1 – beta-site beta-amyloid precursor protein cleavage enzyme 1
B. bifidum – *Bifidobacterium bifidum*
Bax – Bcl-2-associated X protein
BBB – blood-brain barrier
Bcl-2 – B-cell lymphoma 2
Bcl-xL – B-cell lymphoma extra-large
βVLDL – beta-very low-density lipoproteins
BB – bromobenzene
BCG – *Bacillus Calmette-Guerin*
BH4 – tetrahydrobiopterin
BMD – bone mineral density
BPH – benign prostatic hyperplasia
BUN – blood urea nitrogen
Ca^{2+} – calcium
CA1/CA3 – *Comu Ammonis* area 1/3
CAC – coronary artery calcium
CAD – coronary artery disease

cAMP – cyclic adenosine monophosphate
CAT – catalase
CCAAT – cytidine-cytidine-adenosine-adenosine-thymidine
CCl_4 – carbon tetrachloride
CD11b – cluster of differentiation 11b
CD36 – cluster of differentiation 36
cDNA – complementary deoxyribonucleic acid
C/EBP – cytidine-cytidine-adenosine-adenosine-thymidine/enhancer binding protein
cGMP – cyclic 3'5' guanosine monophosphate
CHOP – cytidine-cytidine-adenosine-adenosine-thymidine/enhancer binding protein homologous protein
cIAP1/2 – baculoviral inhibitor of apoptosis repeating-containing protein ½
CML – carboxymethyllysine
cNOS – constitutive nitric oxide synthase
ConA – concanavalin-A
CoQ10 – Coenzyme Q10
COX-2 – cyclooxygenase-2
CP – cyclophosphamide or cisplatin
CPK – creatinine phosphokinase
CRC – colorectal cancer
CSE – cystathionine-gamma-lyase
CYP2E1 – cytochrome P450 2E1
CYP3A – cytochrome P450 isoform 3A
CYS – L-cysteine
CZX – chlorzoxazone
δ-ALA-D –delta-aminolevulinic acid dehydratase activity
DA – dopamine
DADS – diallyl disulfide
DAS – diallyl sulfide
$DASO_2$ – diallyl sulfone
DAT/DATS – diallyl trisulfide
DBP – diastolic blood pressure
DEN – diethylnitrosamine
DG – D-galactose
DGP – dehydrated garlic powder
DHP – 2,6-dimethyl-3-hydroxy-4-pyrone
DiI – 1,1'-dioctadecyl-3,3,3',3'-tetra-methylindocyanide perchlorate
DiI-OxLDL – 1,1'-dioctadecyl-3,3,3',3'-tetra-methylindocyanide perchlorate-labeled oxidized low-density lipoproteins
DMBA – 7,12-dimethylbenz(a)anthracene
DMBA-DNA – 7,12-dimethylbenz(a)anthracene deoxyribonucleic acid
DMH – dimethylhydrazine
DNA – deoxyribonucleic acid
DOX – doxorubicin
DPPH – 2,2-diphenyl-1-picrylhydrazyl
DVT – deep vein thrombosis
EBCT – electron beam computed tomography
EC – endothelial cells
EC_{50} – half maximal effective concentration
E. coli – *Escherchia coli*
ED_{50} – median effective dose
eNOS – endothelial nitric oxide synthase
EPR – electron paramagnetic resonance spectroscopy
ER – endoplasmic reticulum
ERK – extracellular signal-regulated kinase
EU – European Union
F-4 – protein fraction
FD-4 – isothiocyanate-labeled dextran
FMD – flow-mediated endothelium-dependent dilation
Fru-Arg – fructosyl arginine
γδT – gamma delta T (cells)
γ-GluSeMeCys – gamma-glutamyl-selenium-methlyselenocysteine
G_0/G_1 – Gap zero phase/Gap 1 phase

G_2/M – Gap 2 phase/mitosis phase
G6-PD – glucose 6-phosphate dehydrogenase
GFAP – glial fibrillary acidic protein
GLUT2 – glucose transporter 2
GM – gentamicin
GOT – glutamate oxaloacetate transaminase
GPIIb/IIIa – glycoprotein Iib/IIIa
GPE – garlic powder extract
GPT – glutamic-pyruvic transaminase
GPX/GPx – glutathione peroxidase
GR – glutathione reductase
GRP – glucose regulated protein
GSAC – γ-L-glutamyl-*S*-(2-propenyl)-L-cysteine
GSH – glutathione
GSMC – γ-L-glutamyl-*S*-methyl-L-cysteine
GSPC – γ-L-glutamyl-*S*-(trans-1-propenyl)-L-cysteine
GSSG – glutathione disulfide, oxidized glutathione
GST – glutathione *S*-transferase
GST-P – glutathione *S*-transferase pacental form
H_2O_2 – hydrogen peroxide
H_2S – hydrogen sulfide
H. pylori – *Helicobacter pylori*
HAT – histone acetyltransferase
HAT/MET – hypoxanthine, aminopterin, thymidine and methionine
HbA1C – glycated hemoglobin
HC – hemorrhagic cystitis
HCASMC – human coronary artery smooth muscle cells
HCC – hepatocellular carcinoma
Hcy – homocysteine
HDAC – histone deacetylase
HDL/HDL-C – high-density lipoprotein/high-density lipoprotein cholesterol
HGJ – heated garlic juice
H/M – hypoxanthine, aminopterin and thymidine
HMG-CoA – 3-hydroxy-3-methyl-glutaryl-CoA
HNE – 4-hydroxynonenal
HO – heart heme oxygenase activity
HOCl – hypoclorous acid
HODE – hyroxyoctadecadienoic acid
HPLC – high-performance liquid chromatography
HPLC-CIP-MS – high-performance liquid chromatography inductively coupled plasma mass spectrometry
HPN – hippocampal neurons
HPS – hydrogen peroxide scavenging assay
HSC – hepatic stellate cell
HSP70i – inducible 70 kilodalton heat shock protein
IAP – inhibitor of apoptosis
IC_{50} – half maximal inhibitory concentration
ICV-STZ – intracerebroventricular streptozotocin
IFN-γ – interferon-gamma
IgE – immunoglobulin E
IgG – immunoglobulin G
IgG1 – immunoglobulin G1
IgM – immunoglobulin M
IIEF-5 – International Index of Erectile Function with 5 questions
IL-2/6/10 – interleukin-2/6/10
IN – indomethacin
IP – dexamethasone
iNOS – inducible nitric oxide synthase
i.p. – intraperitoneally
I/R or IR – ischemia and reperfusion
JNK – c-Jun N-terminal kinase
JNK1 – c-Jun N-terminal kinase 1

K-Cl – potassium-chloride
KLE – Kyoleopin®
KLEN – Kyoleopin Neo®
L. acidophilus – *Lactobacillus acidophilus*
LC-MS – liquid chromatography-mass spectrometry
LD_{50} – median lethal dose
LE-5 – Leopin-5®
LER – Leopin Royal®
LDH – lactate dehydrogenase
LDL – low-density lipoprotein
LDL-C – low-density lipoprotein cholesterol
LLC – low level chemiluminescence
L-NAME – L-NG-nitroarginine methyl ester
LP – lipid peroxidation
Lp (a) – lipoprotein (a)
Lp-PLA_2 – lipoprotein-associated phospholipase A_2
LPO – lipid peroxidation
LPS – lipopolysaccharides
MAPK/ERK – mitogen-activated protein kinases/extracellular signal-regulated kinases
MAO – monoamine oxidase
MCAO – middle cerebral artery occlusion
MCI – mild cognitive impairment
MCM – conditioned medium from macrophages
MD – mitochondrial dysfunction
MDA – malonaldehyde/malonyldialdehyde
MDA-LDL – malondialdehyde-low-density lipoprotein
MeSeCys – Selenium-methlyselenocysteine
MI – myocardial infarction
MMP-2 – matrix metalloproteinase-2
MMPs-9 – matrix metalloproteinases-9
Mn-SOD – manganese superoxide dismutase
MNU – *N*-methyl-*N*-nitrosourea
MPO – myeloperoxidase
MPP^+ – 1-methyl-4-phenylpyridinium
MPTP – 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/-tetrahydropiridinium
mRNA – messenger ribonucleic acid
MT – microtubule
MTCC/MTCCs – 1-methyl-1,2,3,4-tetrahydro-β-carboline-3 carboxylic acids
MTCdiC – 1-methyl-1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid
mTOR – mammalian target of rapamycin
MTT – methiazol tetrazonium
MTX – methotrexate
NAC – *N*-acetyl cysteine
NADH – reduced nicotinamide adenine dinucleotide
NADPH – nicotinamide adenine dinucleotide phosphate
NAG – *N*-acetyl-β-D-glucosaminidase
NaPB – sodium phenobarbitol
NAPQI – *N*-acetyl-*p*-benzoquinone imine
NCEP – National Cholesterol Education Program
NCI – National Cancer Institute
NSCLC – non-small-cell lung carcinoma
NDEA – *N*-nitrosodiethylamine
NE – norepinephrine
NEDA – *N*-nitrosodiethylamine
NF-κB – nuclear factor kappa B
NGF – nerve growth factor
NIM – neuroendocrine immunomodulation network
NK – natural killer
NMBA – *N*-nitrosomethylbenzylamine
NMOR – nitrosomorpholine
NNK – 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone

NO – nitric oxide
NOC – *N*-nitroso-compounds
NQO1 – NAD(P)H:quinine oxidoreductase 1
NPRO – *N*-nitrosoproline
Nrf2 – nuclear factor erythroid 2-related factor 2
$^1O_2$ – singlet oxygen
O_2^- – superoxide anion
ODC – ornithine decarboxylase
OH^- – hydroxyl radical
•OH – hydroxyl radical
OHCs – organotypic hippocampal slice cultures
$ONOO^-$ – peroxynitrite anion
ORAC – oxygen radical absorbance capacity
OSC – organic sulfur compounds or organotypic spinal cultures
Ox-LDL – oxidized low-density lipoprotein
Ox-PL – oxidized phospholipids
p34 – protein 34
p53 – protein 53
PAC-1 – first procaspase activating compound
PAEC – pulmonary endothelial cells
PAG – propagylglycine
PARP – poly ADP-ribose polymerase
PBS – phosphate buffered saline
PC – protein carbonyl
Pca –prostate cancer
PCNA – proliferating cell nuclear antigen
PCR – polymerase chain reaction
PE – preeclampsia
PG – powdered garlic
PKC – protein kinase C
PKCβ2 – protein kinase C beta 2
PL – phospholipid
PLAC – test for lipoprotein-associated phospholipase A_2
PMA – phorbol 12-myristate 13-acetate
p.o. – per os/oral
PP – pulse pressure
PPARγ – peroxisome proliferator receptor activator gamma
ppb – parts per billion
ppm – parts per million
PPRE – peroxisome proliferator gamma response element
PRP – platelet-rich plasma
PS – porcine serum
PSA – prostate specific antigen
PSMA – prostate specific membrane antigen
PSK – Polysaccharide-K; Krestin
PTPase – protein tyrosine phosphatase
QRS – Q, R and S waves; the combination of three graphical deflections on a typical electrocardiogram (ECG)
RBC – red blood cells
RGJ – raw garlic juice
rHGH – recombinant human growth hormone
ROO• - peroxyl radical
ROS – reactive oxygen species
RT-PCR – reverse transcription-polymerase chain reaction
RW – raw garlic
SAC – *S*-allyl cysteine
SAM – *S*-adenosyl-methionine
SAMC – *S*-allyl mercaptocysteine
SAMP8/SAMP10 – senescence-accelerated mouse prone 8/10
SAMR1 – senescence-accelerated mouse resistant 1
SBC – *S*-benzylcysteine
SCI – spinal cord injury

SDH - succinate dehydrogenase
SDS – sodium docecyl sulfate
Se – selenium
SEC – *S*-ethyl cysteine
SBP – systolic blood pressure
SCH – systemic contact hypersensitivity
SFA – saturated fatty acids
SGC 7901 – human gastric cancer cells line
SHRSP – spontaneously hypertensive stroke prone (models)
Sin-1 – 3-morpholinosydnonimine
SLUG – zinc finger protein (SNAI2)
SMC – *S*-methyl cysteine or smooth-muscle cell
SNAP25 – synaptosomal-associated protein 25
SNP – sodium nitroprusside
SOD – superoxide dismutase
SPC – *S*-propyl cysteine/*S*-propyl-L-cysteine
SPRC – *S*-propargyl-L-cysteine
SREBP-1c/2 – sterol regulatory element-binding protein-1c/2
SS – sulindac sulfide
SSC – skin surface conductance
ST – sulfotransferase
STZ – streptozotocin
TAA – total antioxidant activity
TAG – triacylglycerol
TBA-RS/TBARS – thiobarbituric acid reactive substances
TC – cholesterol or total cholesterol
TEAC – Trolox Equivalent Antioxidant Capacity
TG – triacylglycerol or triglycerides or transglutaminase
tGSH – total glutathione
THB – tetrahydrobiopterin
THβC – tetrahydro-beta-carboline derivatives
TM – tunicamycin
TMP AUC – Post-cuff Deflation Area Under the Temperature Curve
TNF-α – tumor necrosis factor alpha
TPA – 12-O-tetradecanoylphorbol 13-acetate or tetradeanoyl phorbol acetate
TR – temperature rebound
TSOD – Tsumura Suzuki obese diabetes (models)
UDP-GT – uridine diphosphate-glucuronyltransferase
UV – ultraviolet
UVB – ultraviolet B
VEGF – vascular endothelial growth factor
VLDL-C – very low-density lipoprotein cholesterol
$V_v$myo – high volume fraction of myofilament
XIAP – X-linked inhibitor of apoptosis protein
YAC-1 – lymphoma cell line